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Hemibuthus kraepelini, a junior synonym of *Hottentotta rugiscutis* (Scorpiones: Buthidae)

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Abstract. *Hemibuthus kraepelini* Roewer, 1943, is junior synonym of *Hottentotta rugiscutis* (Pocock 1897) The genus *Hemibuthus* Pocock, 1900 has the fixed finger of chelicera with only one ventral denticle, whereas the genus *Hottentotta* Birula, 1908 and examined types of *Hemibuthus kraepelini* have two ventral denticles *Hemibuthus kraepelini* has the same number of granular rows on the movable finger of pedipalps (12), coloration, number of pectinal teeth, overall size, and other characters as *Hottentotta rugiscutis H rugiscutis* is here for the first time placed in the genus *Hottentotta* This species has so far been placed in the genera *Buthus*, *Buthotus* or *Mesobuthus* A key to the Indian species of the genus *Hottentotta* is provided

Taxonomy, new synonymy, key, Scorpiones, Buthidae, *Hemibuthus kraepelini*, *Hottentotta rugiscutis*, India

MATERIAL Designation of the basic trichobothrial pattern (alfa and beta configurations) is adopted from Sissom (1990)

Hottentotta rugiscutis (Pocock, 1897) comb. n.

Buthus rugiscutis Pocock, 1897 106, Kraepelin, 1899 20, Pocock, 1900 26, Takashima, 1945 74
Buthus (Buthus) rugiscutis Roewer 1943 206
Buthotus rugiscutis Vachon & Stockmann, 1968 91
Buthus pachyurus rugiscutis Kraepelin, 1913 130
Buthus rugiscutis nigritus Pocock, 1900 27
Buthus pachyurus nigritus Kraepelin, 1913 130
Mesobuthus rugiscutis Tikader & Bastawade, 1983 229
Hemibuthus kraepelini Roewer, 1943 213 **Syn. n.**

TYPE LOCALITY Mahableshwar Tal, Satara, s Dekhan, India

MATERIAL India, Dekan, Nilgiris, 1 female (hereby designated the lectotype of *Hemibuthus kraepelini*) and 2 males (hereby designated the paralectotypes Nos 1–2 of *Hemibuthus kraepelini*) No 8880/222 in Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main, Germany

DIAGNOSIS The basic trichobothrial pattern is beta (Sissom 1990 70, fig 3 3), the third and fourth legs have well developed tibial spurs, the pectines bear fulcra (Sissom 1990 92, fig 3 17D), the dentate margin of pedipalp-chela movable finger bears distinct granules divided into 12 rows, the entire dorsal surface of the carapace is nearly horizontal in lateral view, the cheliceral fixed finger has two ventral denticles, the second metasomal segment is similar in width to other metasomal segments, the tergites of mesosoma bear three carinae, the carapace bears distinct carinae including central lateral carinae, the trichobothrium *eb* is clearly on the fixed finger of pedipalps, the movable finger of pedipalps bears four principal distal granules and one terminal granule, the first

and second tarsomeres bear paired ventral spines; and the ventrolateral carinae of the fifth metasomal segment have all granules more or less equal in size and never lobate.

Hottentotta rugiscutis is also characterized by yellowish-brown body, pale-yellow legs, total length not exceeding 45 mm, and 20–26 pectinal teeth. Other characters are given in the description of *Hottentotta rugiscutis* (Pocock, 1897: 106).

COMMENTS. Pocock (1897) described *Hottentotta rugiscutis* as *Buthus rugiscutis* from a male and female, which are deposited in the British Museum (Natural History), London, England. In 1900 he further described the subspecies *Buthus rugiscutis nigritus* from one female, also deposited in the British Museum. I refrain from comments on subspecific taxonomy, as they would require more specimens.

Roewer (1943) described *Hemibuthus kraepelini* from one female and two males without selecting one of them as the holotype, without mentioning characters that would justify placing the species in the genus *Hemibuthus*, and differentiating it only from the type species *Hemibuthus crassimanus* (Roewer, 1943: 216).

An enclosed label indicates that the types of *Hemibuthus kraepelini* were examined in 1960 by Max Vachon, who regarted them as *Buthotus kraepelini*, but never mentioned them in print, not even in the revision of the genus *Buthotus* (Vachon & Stockmann, 1968). The genus *Buthotus* Vachon, 1949, is a junior synonym of *Hottentotta* Birula, 1908 (see Fet, 1988: 81).

AFFINITIES. *Hemibuthus* is most closely related to *Hottentotta*. The genus *Hemibuthus* is characterized by only one ventral denticle on the cheliceral fixed finger, whereas *Hottentotta* has two ventral denticles (Sissom, 1990: 97–100).

The four species of the genus *Hottentotta* occurring in India may be differentiated as follows:

1 Pectinal teeth number 26–38. Total length of adult 60–80 mm. ..... *Hottentotta tamulus* (Fabricius, 1798)
– Pectinal teeth number 20–26. Total length of adult 38–50 mm. 2
2 Total length of adult 45–50 mm. Movable finger of pedipalps long, with 13–15 rows of granules. ..... *Hottentotta hendersoni* (Pocock, 1900)
– Total length of adult not more than 45 mm. Movable finger of pedipalps short, with 12 rows of granules . 3
3 Color yellowish-brown. Legs pale yellow, never dark. ..... *Hottentotta rugiscutis* (Pocock, 1897)
– Color dark blackish-green. ..... *Hottentotta pachyurus* (Pocock, 1897)

Acknowledgements

I would like to thank M Grasshoff and Ulrike Schreiber of the Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt am Main, Germany, for the loan of types of *Hemibuthus kraepelini*, and Jiří Zídek for translating the text.

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Vachon M & Stockmann R 1968 Contribution a l'étude des Scorpions Africains appartenant au Genre Buthotus Vachon 1949 et étude de la variabilité *Monitore Zool Ital Firenze* **1968** (2 suppl) 81–149

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BOOK REVIEW

BERGMANN H -H , KLAUS S , MULLER F , SCHERZINGER W , SWENSON J E & WIESNER J 1996 **Die Haselhuhner, *Bonasa bonasia* und *Bonasa sewerzowi*, Haselhuhn und Chinahaselhuhn Die Neue Brehm Bucherei, Band 77** Magdeburg Westarp Wissenschaften, 278 pp , 124 figs, 23 tabs

This monograph presents a detailed review of the available information on two palearctic species of the genus *Bonasa* – *B bonasia* and *B sewerzowi* It is fourth enlargement edition, which brings many new information about species mentioned above The last species of this genus – *B umbellus* from North America is shortly descripted in addition The six authors represent a professional top concerning tetraonids in Europe, especially Dr Klaus is known in the Czech Republic by his investigation of the Hazel Grouse in Šumava Mts , and plans on its reintroduction in Labske piskovce Landscape Protected Area The major attention in presented book is devoted to Hazel Grouse We can find detailed data about its taxonomy, paleonthology, distribution, habitat, behaviour, voice, breeding, food, measurements, weights, ageging, plumage, moults etc I take for valuable chapter concerning present opinions on intraspecific variability including distribution maps of separate subspecies Distribution in Central Europe is worked in great detail Hazel Grouse is here retreating species It lives very inconspicuously and is notoriously difficult to census, therefore indirect methods of its investigation are very important The authors apply here their rich experience in field work, and they devote to this question appreciable attention Capture and ageging methods are descripted very practically a well-arranged The great part of this book is pertained to ecology and behaviour We can find here detailed information about habitat, food composition, results of reintroduction, population biology, diseases, parasites, probable reasons of numbers decrease etc Problems of habitat selection is solved in geographic point of view Very important are conservation proposals for Central Europe – mainly creation of available woodland environment The text concerning behaviour during all the year and breeding biology is supplemented by good black-and-white pictures and photographs Much less of the data is presented to endemic species from restricted area in China and Mongolia, *Bonasa sewerzowi* The least attention was devoted to *Bonasa umbellus* from North America, and concerns mainly intrageneric relations to the two palearctic species, *Bonasa bonasia* and *B sewerzowi* Very useful is also the list of references and subject index

The book can be recommended not only to ornitologists, and specialists interested in the tetraonids, but also to ecologists and people with an interest in nature conservation I believe, that a good knowledge of the ecology nad distribution of threatened animals among the widest public is the important step toward effective protection the animals and their environment

Vladimir Bejček

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Nematode parasites from the blind fish *Ogilbia pearsei* from the Nohoch cave system with remarks on *Rhabdochona kidderi* (Nematoda) from fishes of Yucatán, Mexico

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Abstract. Helminthological examinations of 6 specimens of the rare endemic blind fish, *Ogilbia pearsei* (Hubbs) (Bythitidae), from the Nohoch cave system near Tulum in the Yucatán Peninsula (State of Quintana Roo), south-eastern Mexico, revealed the presence of two species of parasitic nematodes: adults of *Rhabdochona kidderi kidderi* Pearse, 1936 (prevalence 100%, intensity 1–8) and a single encapsulated larva of *Porrocaecum* sp. The morphology of *Rhabdochona kidderi* from *Ogilbia pearsei* proved to be identical with that of nematodes originating from *Rhamdia guatemalensis* (Gunther) (type host), four submedian papilla-like structures on the margin of the oral aperture are described in this species for the first time. The comparison of the *Rhabdochona* Railliet, 1916 specimens from *Ogilbia pearsei* and *Rhamdia guatemalensis* with those from the cenote cichlid *Cichlasoma urophthalmum* (Gunther), collected from the Cenote Zaci, Yucatan, has shown that the nematodes of the last named host species belong to another subspecies, *Rhabdochona kidderi texensis* Moravec et Huffman, 1988, originally described from cichlids in Texas, U. S. A.

Cave fish, Bythitidae, *Ogilbia*, nematode parasites, *Rhabdochona*, *Porrocaecum*, caves, Neotropical Region

INTRODUCTION

Parasites of insular and relict vertebrates have always been of special interest to parasitologists, but there are often fragmentary or no data on the parasites of many relict species and many of them have not yet been examined helminthologically (Stunkard 1970). It concerns also the blind cave fishes occurring in the Peninsula of Yucatán, south-eastern Mexico.

Two endemic species of blind fishes are known from caves and cenotes (= sinkholes) in the Yucatán Peninsula, *Ogilbia pearsei* (Hubbs) (= *Typhlias pearsei* Hubbs) (Bythitidae, Ophidiformes), local name "dama blanca ciega" and the blind eel, *Ophisternon infernale* (Hubbs) (Synbranchidae, Synbranchiformes), local name "anguila ciega Yucateca" (Reddel 1977, Gamboa-Pérez 1992, Espinosa-Perez et al. 1993). Whereas the latter species has not yet been examined for the presence of parasites, there is only one report on the parasites of *O. pearsei* by Chitwood (1938), who recorded the nematode *Rhabdochona kidderi* Pearse, 1936 from the intestine of this fish host from the caves of Yucatan. The exact locality where this parasite was found in *O. pearsei* is not apparent from her paper, she only names four caves (Kaua, Balaam Canche, San Isidro and San Bulha Caves) in the State of Yucatán where *R. kidderi* was recorded from *Rhamdia guatemalensis decolor* Hubbs (Pimelodidae, Siluriformes) and *Ogilbia pearsei*.

Although Chitwood (1938) provided a very short description of *R. kidderi*, it is not clear whether she included specimens from *O. pearsei* in her description or if it was based solely on the specimens collected from its type host, *R. guatemalensis*. Various species of *Rhabdochona* exhibit usually a rather high degree of host specificity (Moravec 1972, 1975) and, therefore, it was important to compare the morphology of *R. kidderi* from *O. pearsei* and *R. guatemalensis* (both these hosts belong to different fish orders and families) to confirm their conspecificity.

In autumn 1994, the authors of this paper obtained through the courtesy of Dr V. E. Urbieta-Ubilla, National Autonomous University of Mexico, a few specimens of *O. pearsei* collected from the Nohoch cave system in the State of Quintana Roo. The results of their parasitological examination are presented herein.

MATERIALS AND METHODS

Six specimens of *Ogilbia pearsei*, body length 4.2–9.1 cm, collected by an American-Mexican Diving Group Corporation from the Nohoch cave system near Tulum, State of Quintana Roo, in September and on 15 October 1994, were examined. The available fishes were fixed in 70% ethanol. After their transfer into distilled water in petri dishes, they were cut open and individual organs were examined under the dissecting microscope. The recovered nematodes were stored in 4% formaldehyde.

In order to be able to compare *Rhabdochona kidderi* specimens from *O. pearsei* with those occurring in *Cichlasoma* spp. in Yucatán, 8 specimens of *Cichlasoma urophthalmum* (Gunther) (Cichlidae, Perciformes) were examined by the second author (J. Vargas-Vázquez) from the Cenote Zaci (20°41'29" N, 88°11'40" W) near Valladolid, State of Yucatán, on 14 July 1995. The freshly collected nematodes were fixed in hot 4% formaldehyde and stored in the same liquid.

For microscopical examination, the nematodes were cleared in glycerine. Drawings were made with the aid of a Zeiss microscope drawing attachment. For scanning electron microscopy (SEM), the nematodes were postfixed in 1% OsO_4, dehydrated through an ethanol series and acetone, and then subjected to critical-point drying. The specimens were coated with gold and examined with a JSM-6300 scanning electron microscope at an accelerating voltage of 15 kV. All measurements are given in millimetres unless otherwise stated. Specimens have been deposited in the Institute of Parasitology, Academy of Sciences of the Czech Republic in České Budějovice and in the Institute of Biology, National Autonomous University of Mexico, Mexico City.

RESULTS

Two species of parasitic nematodes were found in *Ogilbia pearsei*: *Rhabdochona kidderi kidderi* Pearse, 1936 in the intestine of all fishes examined (prevalence 100%, intensity 1–8 [mean 4] nematodes per fish) and a *Porrocaecum* sp. larva encapsulated on the intestinal surface of one fish.

The morphology of *R. kidderi kidderi* from *Ogilbia pearsei* proved to be identical with that of specimens from the type host, *Rhamdia guatemalensis*, from Yucatánese cenotes, as reported by Moravec et al. (1995). SEM micrographs confirmed the presence of 14 anterior teeth in the prostom and bifurcate deirids in this species (Fig. 1); four submedian papilla-like structures at the margin of the oral aperture (Fig. 1 B, D) have been observed in a *Rhabdochona* species for the first time. Measurements of *R. kidderi kidderi* from *O. pearsei* are given in Table 1.

The *Porrocaecum* sp. larva (Fig. 2) is 2.99 long and 0.150 wide, with a very fine transverse striation of cuticle. The cephalic end is blunt. The oesophagus is 0.282 long, the small spherical ventriculus is 0.045 in diameter. The nerve ring and the excretory pore are situated 0.177 and 0.204, respectively, from the anterior extremity. The intestinal caecum is 0.201 long and 0.054 wide. The conical tail is 0.163 long.

Two of eight *Cichlasoma urophthalmum* examined from the Cenote Zaci harboured 3 and 1 specimens of the nematodes biometrically corresponding to the subspecies *Rhabdochona kidderi texensis* Moravec et Huffman, 1988 (Table 1).

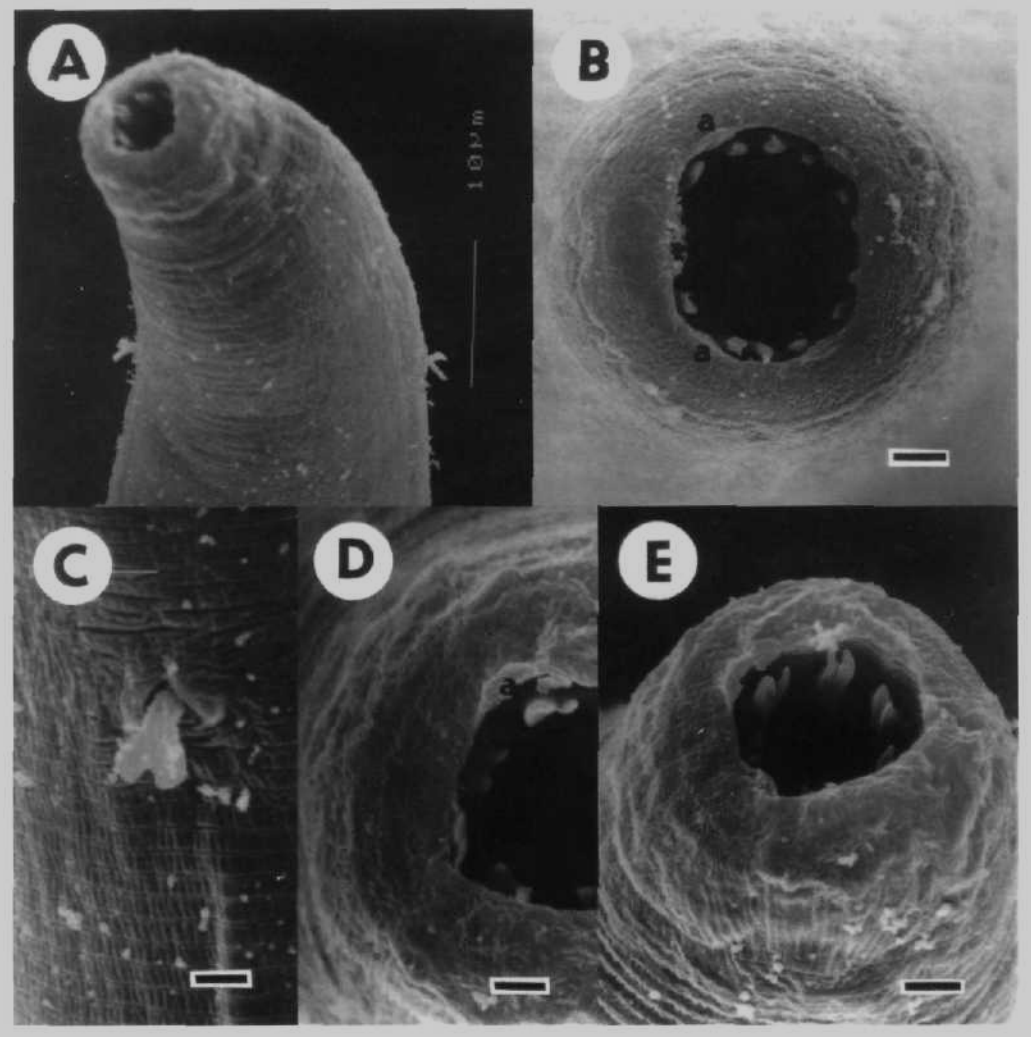

Fig. 1. *Rhabdochona kidderi kidderi* Pearse, 1936 from *Ogilbia pearsei* (Hubbs); SEM micrographs. A – anterior end of body, dorsoventral view (focused on deirids); B – cephalic end, apical view; C – deirid; D – part of oral aperture, apical view; E – cephalic end, subapical view. Abbreviations: a – papilla-like structure at margin of oral aperture. Scale bars: B, E – 2 μm; C, D – 1 μm.

DISCUSSION

The present data show clearly that the nematodes of the genus *Rhabdochona* Railliet, 1916 occurring in the cave fish *Ogilbia pearsei* in the Yucatán Peninsula belong actually to *Rhabdochona kidderi kidderi*, a common intestinal parasite of the catfish *Rhamdia guatemalensis* in Yucatanese cenotes (= sinkholes) and caves (Pearse 1936, Chitwood 1938, Moravec et al. 1995). In contrast to a single finding of a juvenile specimen of this species in *Gambusia yucatana* Regan (Poeciliidae,

Cyprinodontiformes), reported by Moravec et al. (1995), the nematodes from *O. pearsei* were mostly mature, including females with fully mature eggs. This indicates that *O. pearsei* acts as the true definitive host of *R. kidderi* in which the nematode attains the maturity and can reproduce; the high prevalence of *R. kidderi* in *O. pearsei* supports this view.

Since various species of *Rhabdochona* are known to exhibit a rather high degree of host specificity, the situation that *R. kidderi* utilizes as its definitive hosts fishes belonging to different fish orders (Siluriformes and Ophidiformes) is noteworthy. Because both *O. pearsei* and *R. guatemalensis* occur in the same habitats (cenotes and caves) with little diversified ecological conditions, it may well be that this reflects in the decrease of the degree of host specificity in *R. kidderi* (a similar phenomenon was observed in the populations of some *Rhabdochona* species near the border of their distribution area – Moravec 1975), although it cannot be excluded that catfishes (*R. guatemalensis*) serve as the so called postcyclic hosts of *R. kidderi*, acquiring infection of mature nematodes while feeding on infected true definitive hosts, *O. pearsei*. Another explanation may be the fact that *Rhabdochona* spp. are known to be capable of the precocious development in the body of the insect intermediate host (Gustafson 1942, Moravec 1976, 1994), where they may achieve maturity and are, sometimes, even ready to produce eggs almost as soon as they reach the gut of the fish host (Anderson 1988). Unfortunately, nothing is known at present about the life cycle of *R. kidderi*.

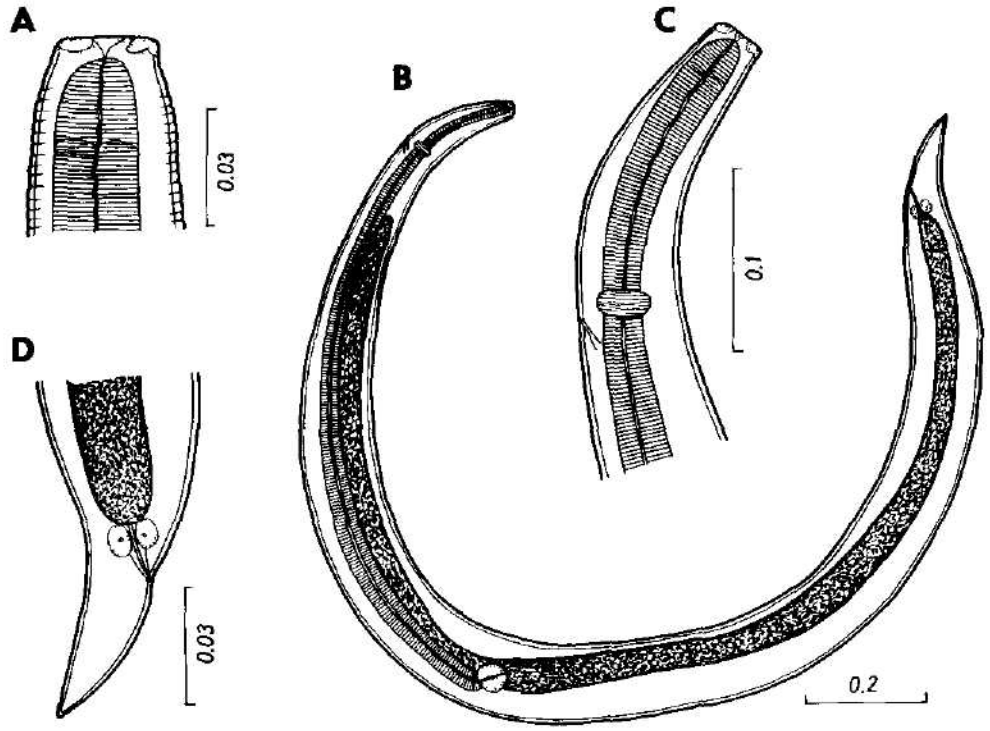

Fig. 2. *Porrocaecum* sp. larva from *O. pearsei* (Hubbs). A – cephalic end; B – general view; C – anterior end of body; D – tail. Scale bars in mm.

The morphology of *R. kidderi kidderi* from *O. pearsei* is in a full accordance with the existing descriptions of this species based on specimens from its type host, *R. guatemalensis* (see Pearse 1936, Chitwood 1938, Gustafson 1949, Moravec & Arai 1971, Moravec et al. 1995). The SEM study has shown, for the first time, the presence of four submedian papilla-like structures on the margin of the oral aperture; similar structures seem to be absent in some other congeneric species.

In 1988, Moravec & Huffman (1988a) described conspecific nematodes from *Cichlasoma cyanoguttatum* (Baird et Girard) and *Tilapia mossambica* (Peters) (both fam. Cichlidae) (juvenile forms also from *Gambusia affinis* (Baird et Girard) (Poeciliidae) from Texas, U. S. A., for which they had established an independent subspecies, *Rhabdochona kidderi texensis*. In contrast to the nominotypical subspecies, *R. kidderi texensis* has a markedly longer left spicule (about 2 mm), different length ratio of spicules and somewhat smaller eggs. Later this subspecies was reported from *Cichlasoma fenestratum* (Günther) from Lake Catemaco, Veracruz, Mexico (Pérez-Ponce de León et al. 1996). The present material of *R. kidderi texensis* from *Cichlasoma urophthalmum* more or less agrees with the original description of this subspecies. Although in the Yucatán Peninsula *C. urophthalmum* frequently occurs in cenotes along with *R. guatemalensis*, the type host of *R. kidderi kidderi*, the specimens of *Rhabdochona kidderi* from these two host species distinctly differ in the length of their left spicule, which in specimens from *Cichlasoma* is approximately twice as long as that in those from *Rhamdia* and *Ogilbia*.

The finding of the encapsulated *Porrocaecum* sp. larva in *O. pearsei* indicates that this fish serves probably as the paratenic host of this nematode species. Adults of this genus are intestinal parasites of birds and, therefore, it is hardly possible to expect that the fish became infected in the cave; more probably the fish acquired the infection in a cenote from where it migrated into the cave. Cenotes or sinkholes are small surface, mostly freshwater bodies connected with subterranean streams; there are several records of *O. pearsei* from cenotes in the Yucatán Peninsula (Reddel 1977).

It is interesting that species of *Rhabdochona* are so far the only known adult nematodes reported from blind fishes from the subterranean waters. In addition to *Rhabdochona kidderi* from *Ogilbia pearsei* in Mexico, another congeneric species, *Rhabdochona longleyi* Moravec et Huffman, 1988, a specific parasite of the toothless blindcat, *Trogloglanis pattersoni* Eigenmann, and the widemouth blindcat, *Satan eurystomus* Hubbs et Bailay, both members of the family Ictaluridae, Siluriformes, was described from the artesian wells near San Antonio, Texas, U. S. A. (Moravec & Huffman 1988b). Unfortunately, nothing is known about the life histories of these *Rhabdochona* species parasitizing fishes living in the subterranean waters. Moravec & Huffman (1988b) suggested that *R. longleyi* probably adapted to the environment of the subterranean waters by utilizing available crustaceans instead of insects as its intermediate hosts. But it may well be that *R. kidderi*, occurring in both caves and cenotes, can develop through both aquatic insects and crustaceans, similarly as some species of the related fish nematode genus *Spinitectus* Fourment, 1883 (Jilek & Crites 1981).

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Life-histories and the description of developmental stages of *Theridion bimaculatum*, *T. impressum* and *T. varians* (Araneae: Theridiidae)

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Abstract. Life-histories of the three most abundant theridiid spiders of an apple orchard (*Theridion varians* Hahn 1833, *T. impressum* L. Koch 1881, and *T. bimaculatum* (Linnaeus 1767)) are presented. The juvenile and subadult instars are described in detail. The instars were identified according to the length of tibia and metatarsus of leg I, and number of hairs in medial and submedial rows of both tibia and metatarsus. Five juvenile and two subadult instars were recognized in each of the species studied. The colour pattern of each stage is given. The results obtained are a sound basis for further population analysis, such as construction of life-tables.

Life-history, development, key, orchard, Araneae, *Theridion*, Czech Republic

INTRODUCTION

The study of the population dynamics of spiders has attained more interest in recent years (e. g., Topping & Sunderland 1994). Particularly, since it is recognized that spiders are one of the most important predators of many invertebrate pests. Such studies enable us to understand population processes in order that we might be able to predict changes in the populations of spiders.

The adult stages are, in some spider species, very well defined and, therefore, those species are easily identified. However, even in some common spiders, immature individuals are difficult to identify to a species level. Thus many authors have preferred to leave these stages unidentified or assigned them only to genus level. Identification of all developmental stages into species not only increases our knowledge but in particular enables us to construct life tables which are a sound basis for further population analysis (Houseweart & Kulman 1976).

This paper deals with the description of juvenile and subadult stages of the three most abundant species of theridiid spiders (*Theridion varians*, *T. impressum*, and *T. bimaculatum*) that were considered as principal agents of pests in a studied orchard. Though there was a considerable number (more than 100) of life-history studies performed on spiders, this is in fact negligible considering the number of spider species. Moreover, only a few of them gave a detailed description of stages of development, leaving great scope for further study before we can safely identify even very common species.

MATERIAL AND METHODS

The spiders were collected during 4 years (1992–1995) of intensive research in an apple orchard in Horoměřice near Prague (Czech Republic). Samples were taken by means of tapping the low branches of apple trees over trays, sweeping weed strips and hand collecting females with eggsacs (to identify the earliest instars). Altogether 2549 individuals of the genus *Theridion* (*T. varians*, *T. impressum* and *T. bimaculatum*) were collected. Out of these 180

Tab 1 *Theridion bimaculatum* (Linnaeus): mean length of Ti and Mt (±standard deviation), Mt Ti ratio, and number of hairs the medial (Ti1, Mt1) and submedial (Ti2, Mt2) rows of Ti and Mt in the particular stages Abbr see in the text

	length (mm)		ratio	no. of hairs			
	Ti	Mt	Mt : Ti	Ti1	Mt1	Ti2	Mt2
J1	0 17 ±0 01	0 16 ±0 01	0.94	4	4	4	4
J2	0 23 ±0 01	0 23 ±0.01	1.00	5	5	5	5
J3	0 27 ±0 02	0 27 ±0 02	1.00	6	6	6	6
J4	0 32 ±0 02	0.32 ±0.02	1.00	7	7	7	7
J5	0 37 ±0 03	0.37 ±0 02	1 00	8	8	8	8
S1	0 59 ±0.12	0 62 ±0.12	1.05	8–9	9–10	8–10	9–10
S2	1 08 ±0.17	1 07 ±0 15	0.99	10–14	11–16	9–14	10–16
M	2 18 ±0 54	2 30 ±0 56	1.05	16–22	20–27	15–20	18–24
F	1 64 ±0.23	1 70 ±0 25	1 04	12–16	14–19	11–15	13–17

individuals of *T varians*, 182 individuals of *T impressum* and 123 individuals of *T. bimaculatum* were examined using a binocular microscope. The following criteria for discrimination were followed:
- length of tibia (Ti) of left leg I
- length of metatarsus (Mt) of left leg I
- number of hairs in a medial row of tibia (Ti1) of left leg I on the prolateral side (Fig 1)
- number of hairs in a submedial row of tibia (Ti2) of left leg I on the prolateral side (Fig 1)
- number of hairs in a medial row of metatarsus (Mt1) of left leg I on the prolateral side (Fig 2)
- number of hairs in a submedial row of tibia of metatarsus (Mt2) of left leg I on the prolateral side (Fig 2)
- colour pattern of left leg I (Fig 8)
- colour pattern of clypeus and chelicera (Fig 3)
- colour pattern of carapace
- colour pattern of sternum
- colour pattern of upper surface of abdomen (Fig. 4, 9, 10, 12)
- colour pattern of lower surface of abdomen (Fig. 11, 13)

The first six (i e numeric) characters were first analyzed using K-means clustering (an extension of Cluster analysis in STATISTICA package). A goodness of classification into defined stages was tested by Discriminant analysis and 1-way ANOVA In all three species 5 juvenile (J1. J5), 2 subadult (S1, S2), and 2 adult (M, F) instars were recognized.

RESULTS

Theridion bimaculatum (Linnaeus, 1767)
(Figs 1, 2, 5, 12, 13)

Observed characters of the instars are displayed in Table 1. The differences between stages were highly significant [$P < 0.001$; LSD tests in ANOVA] in the length of Ti and Mt. The number of hairs varies between instars from J5. Both length of Mt and Ti increase identically ($slope_{Mt} = 0.25$, $slope_{Ti} = 0.24$). However the number of hairs of Mt increases at a considerably higher rate than on Ti ($slope_{Mt1} = 2.16$, $slope_{Mt2} = 2.04$, $slope_{Ti1} = 1.76$, $slope_{Ti2} = 1.60$). Both Ti and Mt are longer in males than in females in adults, and the number of hairs in males is also greater. In all instars the length of Ti is very close to the length of Mt.

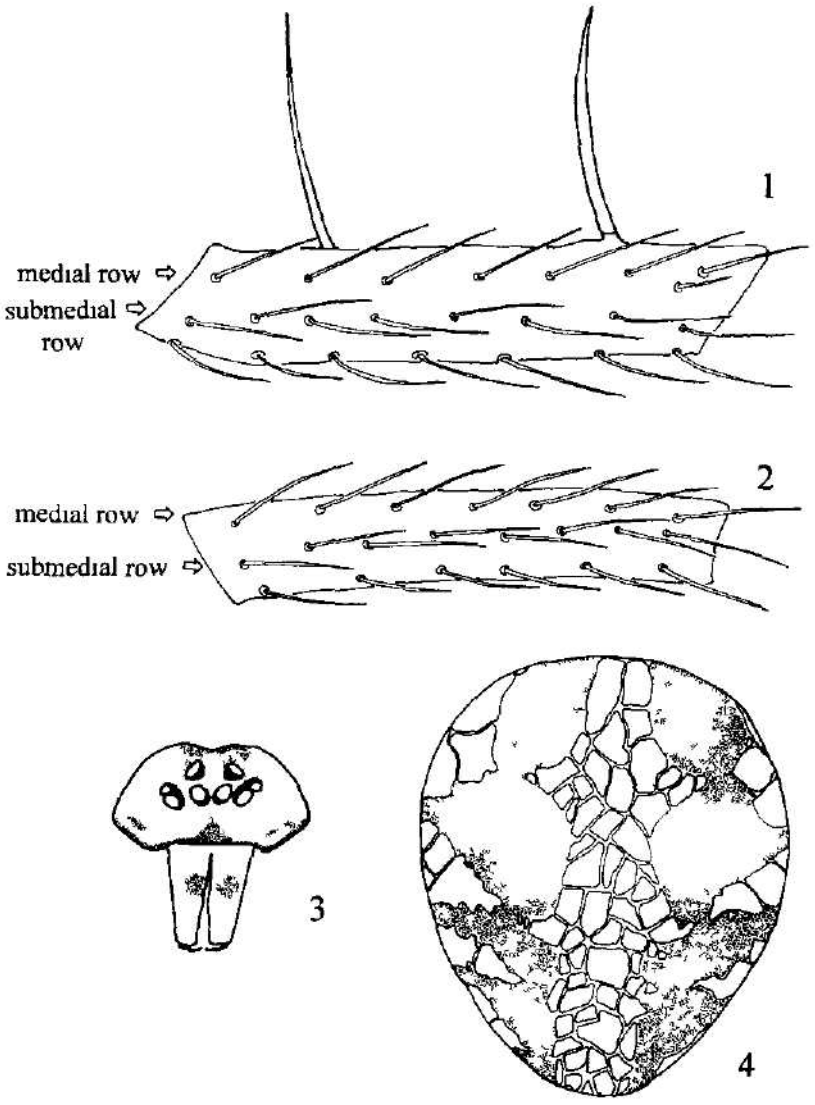
The colour pattern of this species changes a little from juvenile to subadult. The body colour is yellow and dark brown.

Clypeus and chelicerae: In all stages without any marking.

Carapace: In later stages uniformly brown; in J1– J3 there is only a brown band in the midline.

Sternum In adult spiders uniformly brown whereas in the first two instars uniformly yellow, later with separate brown markings along margin

Abdomen The dorsal surface in the first two instars yellow or slightly brown with a rather large, compact white marking centrally (Fig 12), in later stages dark brown areas occur on the lateral



Figs 1–4 1 – *T bimaculatum* (Linnaeus), tibia of leg I and the position of hairs in medial and submedial row, 2 – *T bimaculatum* metatarsus of leg I and the position of medial and submedial row, 3 – *T varians* Hahn, clypeus and chelicera (J4), 4 – *T varians* upper surface of abdomen (J3)

Tab 2 *Theridion varians* Hahn: mean length of Ti and Mt (±standard deviation), Mt:Ti ratio, and number of hairs in the medial (Ti1, Mt1) and submedial (Ti2, Mt2) rows of Ti and Mt in the particular stages Abbr see in the text

	length (mm)		ratio	no of hairs in			
	Ti	Mt	Mt : Ti	Ti1	Mt1	Ti2	Mt2
J1	0 22 ±0 01	0 21 ±0 01	0.95	4	4	4	4
J2	0 30 ±0 02	0 31 ±0 03	1 00	5	5	5	5
J3	0 35 ±0 02	0 37 ±0 02	1.06	6	6	6	6
J4	0 47 ±0 02	0 50 ±0 03	1.06	7	7–8	7	7–8
J5	0 54 ±0 03	0.57 ±0 04	1.06	8–9	9–10	8–9	9–10
S1	0 70 ±0 07	0 77 ±0 09	1.10	9–11	11–13	9–11	10–13
S2	0.94 ±0 09	1 05 ±0.13	1.12	10–12	12–15	10–12	11–14
M	1.61 ±0 38	1 86 ±0 50	1.15	10–13	14–18	11–14	14–17
F	1 64 ±0 11	1 88 ±0 17	1 14	10–13	13–16	10–13	11–15

margins of abdomen; finally, there are two forms of pattern: uniformly brown (usually in males) or with a white central band and more or less brown margin (usually in females); the ventral surface in J1 and J2 lacks any pattern but in later instars has a broad brown marking in the centre (Fig. 13), finally becoming uniformly brown.

Legs: Pale in all juvenile and subadult instars and in the adult female, however in adult males they are yellow. There are no markings or bands in any immatures and adults.

The phenology diagram is displayed in Fig. 5. In 1994 this species overwintered in J3–J5 instars. Males reached maturity in May and females in June. Males died out by the end of July whereas females carried on until August. The first spiderlings occurred in July, 1994.

Theridion varians Hahn, 1833
(Figs 3, 4, 6, 8)

Observed characters of the recognized instars are given in Table 2. The differences between each instar were highly significant [$P < 0.003$; LSD test in ANOVA] in the length of Ti and Mt. The number of hairs in the studied rows of Ti and Mt is conspecific in J1–J4, but overlaps in latter stages. The Mt length increases from J1 to adult at a slightly higher rate than the length of Ti ($slope_{Mt} = 0.21$, $slope_{Ti} = 0.18$). The same trend is found in the number of hairs on Mt and Ti (cf. $slope_{Mt1} = 1.61$, $slope_{Mt2} = 1.42$, $slope_{Ti1} = 1.09$, $slope_{Ti2} = 1.11$). The length of Ti equals the length of Mt in J1–J5, but from S1 Mt becomes longer than Ti so that in adult stage Mt is 1.15 times longer than Ti.

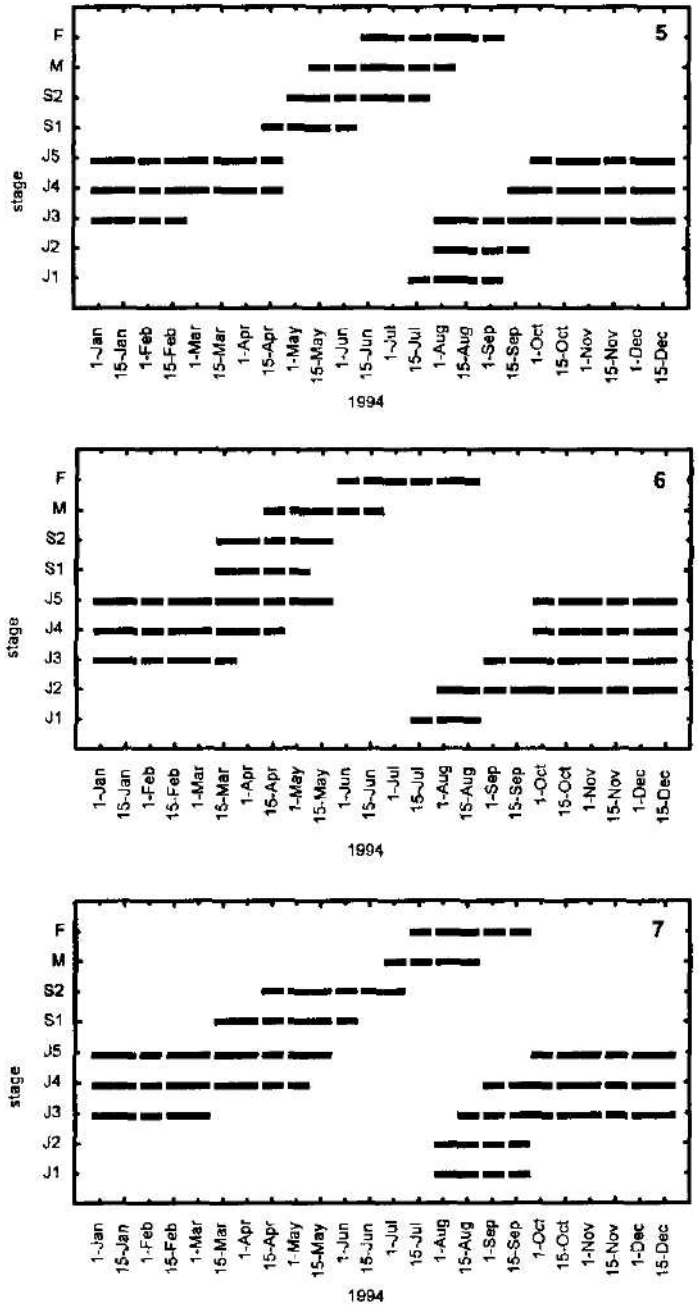
The colour pattern of this species changes only slightly during development, so the appearance of an immature is very similar to an adult spider. The body colour in this species is pale-yellow with black and gray markings or bands.

Clypeus and chelicerae: In all instars there is a clear black triangular marking on the clypeus, and, from J3, a pair of black markings appears near the base in front of chelicera (Fig. 3).

Carapace: All instars have a well defined black band in the midline and sometimes darker broad margins as in adults; the margins are less clear or missing in the first instar.

Sternum: In most instars this has a broad black margin as in adults; in J1..J3 the margins are thinner or reduced to separate markings.

Abdomen: The dorsal surface has two distinct forms: uniformly black with a white marking situated anteriorly, or black/white pattern forming two parallel bands (Fig. 4); both forms were observed evenly throughout all stages and sexes; the lower surface is in J1 and J2 without any marking, later with centrally oriented black bands.



Figs 5 – 7. Phenology diagrams, 5 – *Theridion bimaculatum* (Linnaeus) (Horoměřice, 1994); 6 –*T. varians* Hahn (Horoměřice, 1994); 7 – *T. impressum* L. Koch (Horoměřice, 1994).

Tab 3 *Theridion impressum* L Koch· mean length of Ti and Mt (±standard deviation), Mt·Ti ratio, and number of hairs in the medial (Ti1, Mt1) and submedial (Ti2, Mt2) rows of Ti and Mt in the particular stages Abbr. see in the text

	length (mm)		ratio	no. of hairs			
	Ti	Mt	Mt Ti	Ti1	Mt1	Ti2	Mt2
J1	0 23 ±0 01	0 26 ±0 01	1 13	4	4	4	4
J2	0.26 ±0 01	0 30 ±0.02	1.15	5	5	5	5
J3	0 32 ±0 01	0.36 ±0.03	1.13	6	6	6	6
J4	0 36 ±0.02	0.43 ±0.03	1 19	7–8	7–8	7–8	7–8
J5	0 41 ±0 03	0.50 ±0.03	1 22	9–10	9–10	9–10	9–10
S1	0 56 ±0 04	0 72 ±0 09	1.29	9–11	10–13	9–11	10–13
S2	0 89 ±0 12	1 19 ±0.22	1.34	9–14	13–19	9–12	12–18
M	1 50 ±0 17	2.08 ±0.28	1 39	13–16	19–25	11–18	16–23
F	1 59 ±0 12	2.17 ±0 15	1 36	12–16	18–23	11–15	16–22

Legs: In the early instars only the extreme ends of tibiae and metatarsi are darkened, in later instars and adults more black markings (at least 3 per segment) occur throughout leg segments (Fig. 8).

The phenology is shown in Fig. 6. In 1994 this species overwintered as juveniles of all instars except J1. Males become adult in April but females did not mature until the beginning of June. Males died out by the end of June whereas females lived until August. The first spiderlings occurred in July, 1994.

Theridion impressum L. Koch, 1881
(Figs 7, 9–11)

Observed characters of the recognised stages are given in Table 3. The differences between stages were highly significant [$P < 0.001$; LSD test in ANOVA] in the length criteria. The number of hairs vary considerably from instar J4. The length of Mt increases at a higher rate than the length of Ti ($slope_{Mt} = 0.25$, $slope_{Ti} = 0.17$). The number of hairs of Mt thus also increases at higher rate than on Ti ($slope_{Mt1} = 2.33$, $slope_{Mt2} = 2.00$, $slope_{Ti1} = 1.37$, $slope_{Ti2} = 1.25$). Although both Ti and Mt are longer in adult females than adult males, the number of hairs is greater in males. In all instars Mt is longer than Ti, and in adult stages it is nearly 1.4 times longer than Ti. In this species the Mt:Ti ratio changes dramatically from instar to instar.

The colour pattern of this species changes slightly during development. The body colour of this species is yellow with brown markings or bands.

Clypeus and chelicerae: Without markings in any immatures and adults.

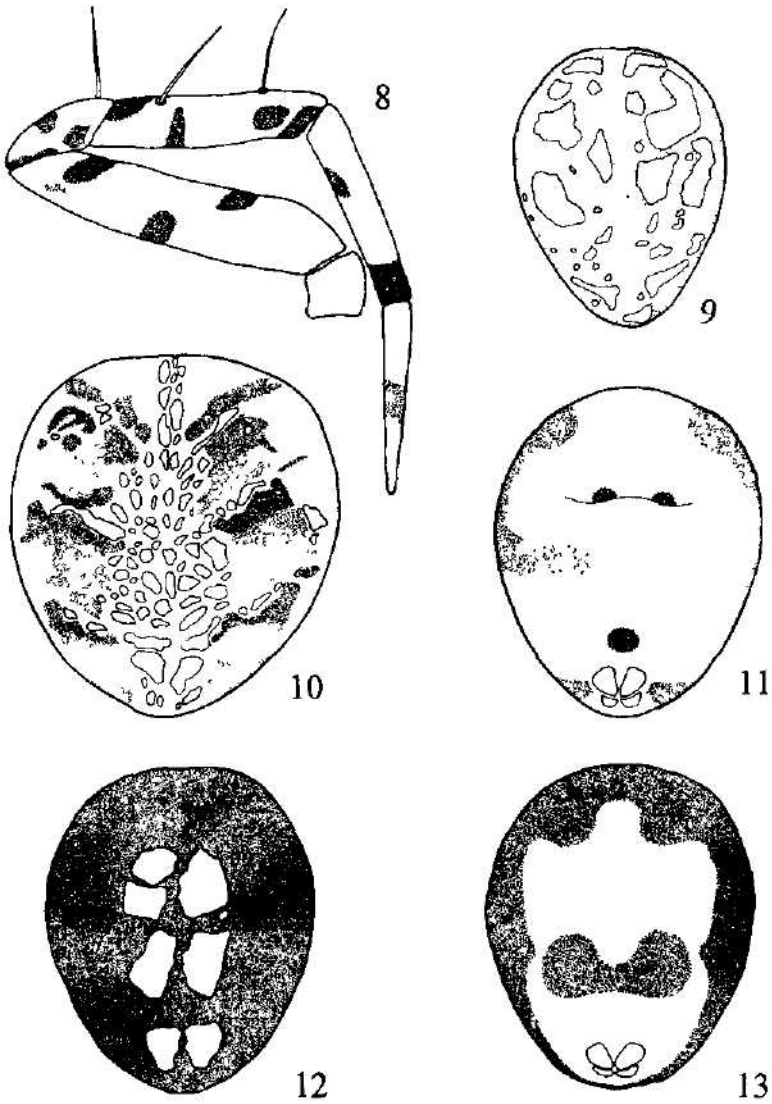
Carapace: From instar J3 there is a well defined dark-brown band in the midline and possibly thin darker margins; in instars J1 – J3 the margins are missing or very thin.

Sternum: In instars J1 and J2 there are no markings. After that there is a brown band.

Abdomen: In the first two instars only the dorsal surface has dispersed white markings (Fig. 9); in later stages the number of white and brown markings increases and forms two parallel bands (Fig. 10); the ventral surface in nearly all instars with a small brown marking in front of spinners (Fig. 11); in later stages (from J3) there is a pair of brown markings in the epigastric region.

Legs. There are no markings in J1 and J2, later the distal end of each segment is darkened, and in the adult there is a marking in the middle of Ti and Mt.

The phenology diagram is displayed in Fig. 7. In 1994 this species overwintered as instars J3–J5. Males became adult in early July and females towards the end of July. Males died out in August and females in September. The first spiderlings occurred in August, 1994.



Figs 8–13 8 – *T. varians* Hahn, left leg 1 (S1); 9 – *T. impressum* L. Koch, upper surface of abdomen (J1); 10 – *T. impressum* L. Koch, upper surface of abdomen (J5); 11 – *T. impressum*, lower surface of abdomen (J3); 12 – *T. bimaculatum* (Linnaeus), upper surface of abdomen (J3), 13 – *T. bimaculatum*, lower surface of abdomen (J3)

Key to identification of the earliest instars of the studied species

1. clypeus and possibly chelicera with a black marking (Fig. 3), whole body pale with black markings. . . . . *T. varians*
– clypeus and chelicera without any markings, body mostly yellow 2

2. dorsal surface of abdomen with many small white spots (Fig. 9) dispersed over whole surface, whole body yellow ..... *T. impressum*
– upper surface of abdomen yellow with a compact white band along the midline (Fig. 12) or completely brown, whole body yellow to brown ..... *T. bimaculatum*

DISCUSSION

Developmental stages of the three most abundant theridiid spiders occurring in the apple orchard were studied. All the species, *Theridion varians*, *T. impressum*, and *T. bimaculatum* have very similar biennial cycle; reproduce in summer, spiderlings emerge about a month later and overwinter as juveniles. The aim of this study was to find such characters that would best identify the particular developmental stages. Previously such discrimination of stages was carried out using various external morphological characters, such as breadth or length of carapace (Dondale 1961, Blandin & Célérier 1987), trichobothriotaxy (Canard 1987, Ysnel 1988), number of teeth on tarsal claws, or on chelicera (Bonnet 1930), and the length of the tibia of the first leg (Toft 1976). In the presented study, the length of tibia and metatarsus (leg I) and the number of hairs in medial and submedial rows of Ti and Mt were selected as the principal indicators. As noted by Toft (1976), the length of Ti provide a much more clear-cut separation of instars than cephalothoracic measurements. Linear numeric characters appeared to be suitable because they changed considerably from instar to instar and showed only small variation within each instar. The variation increased in older stages as a result of unequal development. A similar variation was observed quite frequently, by e. g., Ysnel (1988), in number of trichobothria of *Larinoides cornutus*, or by Dondale (1961) in breadth of carapace of several species.

The three species studied are easily distinguished from each other as adults not only by the shape of male palpus or female epigyne but also on basis of colour pattern. Since it is generally believed that the colour pattern changes during development some doubts about the identification of juvenile or subadult instars might occur. Surprisingly, in the studied species the pattern did not differ considerably between stages and some patterns were found to be specific to a species. Only in the earliest juvenile instars, J1 and J2, is the colour pattern different because the specimens are not fully pigmented yet. However, even in these stages presence of certain patterns enables safe identification to species.

There were at least 5 to 9 juvenile instars observed during several investigations (Ysnel 1991, Blandin & Célérier 1986, 1987). Inconstancy in the number of developmental instars was reported by many authors (e. g., Dondale 1961) and is believed to be linked to variation in environmental conditions (Ysnel 1991). In the presented study 7 juvenile stages were recognized, however, it is expected that the number might be higher. Since the species were not reared but collected without knowledge of their life-history it was not possible to state the number more precisely. A similar problem was encountered in the classification of adults. Ysnel (1991) pointed out that the final adult stage can be reached from several juvenile instars. A very large variation in the length of Ti and Mt and in the number of hairs in both adult sexes reveal that this stage might have been reached from different instars. However, collected specimens did not provide sufficient basis for further differentiation, so all adults were classified into one stage.

It was also difficult to state at what stage the juvenile of the studied species became free-living Because it is suggested by Downes (1987) that most specimens of *Theridion rufipes* Lucas emerge from the cocoon as first instar spiderlings, I considered the smallest free-living spiderlings as the first instar.

Toft (1976, 1978) centred his study upon life-histories of a number of spiders, including *T. varians*. He also classified immatures into instars according to the length of tibia of the first leg, but

separated only 5 instars. Unfortunately, he does not present observed criteria of the particular instars. Since he could recognize sex from the third instar (by swellings of palpus in males and the epigynal area in females), it appears that his stage III is identical to S1/S2 in this study. Despite the differences in the number of instars, life-history of *T. varians* in Denmark is very similar to that in the Czech Republic.

This study attempted to give an accurate definition of life-history stages. These might differ from results obtained in breeding experiments. Thus some interpretations might not be correct. However, significant differences between the defined stages support the results of this study.

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BOOK REVIEW

CLEMENTS A N **The Biology of Mosquitoes. Volume 2 Sensory Reception and Behaviour** Wallingford, Oxon CABI Publishing, a division of CAB International, 1999 XV + 740 pages Format 185×245 mm *Hardbound, price Lstg 95 00 (USD 175 00) ISBN 0 85199 313 3*

The author is a professor affiliated with the London School of Hygiene and Tropical Medicine This is the second of the three projected volumes of *The Biology of Mosquitoes* Volume 1 published by Chapman and Hall in 1992 focused on development, nutrition and reproduction, growth and metamorphosis of larval and adult forms of mosquitoes, egg production by the adults females and physiological adaptations of larvae to their aquatic environment This second title provides information on the physiology of mosquitoe sensory organs and behavioural patterns In the introduction looked at are biological cycles, endogenous rhythms, dial and lunar periodicities in mosquitoe behaviour The volume is composed of 17 chapters numbered in consequence of the preceeding volume 1 from 24 through 40

Chapter 1 (24) describes integumental sensilla of culicid larvae comprising articulated setae of the body surface, antennal and maxillary sensilla Following chapter is dedicated to adult integumental sensilla their structure, physiology and connections with the brain Chapter 3 concentrates on antennae and hearing, in particular on structure of the antennae, chordotonal organs, Johnston's organ, antennal fibrillae, sound signals and hearing Chapters 4 and 5 (27 and 28) deal with larval and adult eyes and vision The chapter 6 on behaviour of larvae and pupae characterizes aquatic environment in relation to locomotion, taxis and kinesis, escape from predators, feeding behaviour, grooming and various other activities Following chapters highlight adult circadial rhythms and the regulation of adult behaviour, including regulation of male and female sexual activities, responsiveness to host cues, oviposition and feeding Chapters 9 and 10 (32 and 33) lay emphasis on the modification of adult behaviour by geophysical and climatic factors and the flight with respect to anatomical structure and function of wings, flight capability and orientation in relation to wind, the hights at which mosquitoes fly, migration, and more Next two chapters provide coverage of male and female genitalia and associated organs and the mating with reference to various genera and species of mosquitoes

Chapter 13 (36) deals with feeding on plant sugars Chapters 15 and 16 (38 and 39) are concerned with sources and characteristics of host cues and with host finding, namely with reactions of mosquitoes to chemical, physical and visual stimuli and with behavioural patterns exhibited by mosquitoes responding to host over a distance Chapter 16 (39) analyses mosquitoe-host interactions, with particular emphasis on the host specificity and defensive responses of the host, blood-meal identification by serological methods, factors determining host feeding patterns, and transmission of parasites The concluding chapter is devoted to detailed analysis of behavioural events leading to egg laying by *Anopheles, Culex, Culiseta, Aedes, Mansonia, Coquillettidia* and other mosquitoe genera

The volume concludes with a comprehensive list of references and is augmented by a wealth of figures numbered by individual chapters, composed of schematic line drawings, photographs and graphs In addition, there are numerous tables summarizing data given in the textual part Eleven chapters contain thematic glossaries of terms relating to blood feeding, host finding, mating, oviposition, rhythmic and cyclic behaviour and other terms

Mosquitoes are important transmitters of widespread major diseases as are malaria and arboviruses They are also one of the most studied and well-known group of insects, both in the laboratory and in the field This volume offers an in-depth review of biology of sensory organs and environmental relations influencing the vertebral host attack by mosquitoes Following volume 3 in preparation, approximate publication date 2004, is intended to inform on dormancy, survival, speciation and evolution

Jindřich Jira

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Dicerca bilinica sp. n., a new species of buprestid-beetle (Coleoptera: Buprestidae) from Lower Miocene of the Most formation in northern part of the Czech Republic

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Abstract. A new fossil species of Buprestid beetle, *Dicerca* (s. str.) *bilinica* sp. n. from Lower Miocene deposits in Bílina brown coal mine, is described and compared with both recent and fossil representatives of the genus *Dicerca* Eschscholtz, 1829

Taxonomy, fossil, Buprestidae, *Dicerca bilinica* sp. n., Miocene, Central Europe

INTRODUCTION

Tertiary beetle founds from north Bohemian basins are known from late nineteenth century papers from localities of the Kučlín (Upper Eocene; Deichmüller 1881), Lužice (Upper Oligocene; Beier 1952), Mokřina (Lower Miocene; Novák 1877) and Pochlovice u Knšperka nad Ohří (Upper Oligocene; Ponomarenko 1973, Říha 1961, 1974). A new quarry with uniquely preserved insect fauna has been discovered recently at Bílina mine in northern Czech Republic by palaebotanists and Zdeněk Dvořák (local collector), Prokop (1998). The insects are preserved in three different fossiliferous horizons (Lake Clayey Horizon, Delta Sandy Horizon, Clayey Superseam Horizon) dated to Lower Miocene (Eggenburgian/Ottnangian). Reconstruction of palaeoenvironment in Delta Sandy Horizon by palaeobotanists shows alternation of calm lake sedimentation and meandering river arms sedimentation (Bůžek et al. 1992). The layers belonging to Delta Sandy Horizon are usually dominated by *Salvinia reussi* Ett., typical floristic element of aquatic environment (Kvaček 1998). Palaeobotanical record suggests warm temperate to subtropical climatic conditions (Fejfar & Kvaček 1993).

The genus *Dicerca* Eschscholtz, 1829 is very rare in fossil records. There are only a few European specimens described from Middle Eocene (Hörnschemeyer & Wedmann 1994, Wedmann & Hörnschemeyer 1994), Upper Miocene (Heer 1847) and from Upper Oligocene (Assmann 1870, Heyden 1856, 1859). In a recent fauna the genus comprises 14 species in Palearctic region and about 70 species in the world fauna.

MATERIAL AND METHODS

One imprint No. ZD9704 was obtained from the paleontological collection of Zdeněk Dvořák (Doly Bílina) and it is deposited in the collection of the Bílina mine, Czech Republic. The specimen consists of the head with fragments of compound eyes, pronotum partly covered by sediment close to right lateral margin, fragments of left fore, both middle and left hind legs, elytra better preserved in distal part, apex of abdomen visible between opened elytra. The specimen is preserved in fine-grained light-brown claystone of Delta Sandy horizon, fossiliferous horizon No 24. The specimen was collected in 1992 by Z. Dvořák and determined by authors.

Drawing and photograph were obtained by using of classical methods with stereomicroscope. Fossil specimen was kept in dry state

TAXONOMIC PART

Dicerca (s. str.) *bilinica* sp. n.
(Figs 1, 2)

DESCRIPTION. Body large, subparallel, pronotum distinctly narrower than elytra.

Head convex, vertex 1. 8 times as wide as width of eye; eyes not projecting beyond outline of head; sculpture of head consisting of small, rounded and dense punctures; antennae and mouth parts not preserved.

Pronotum widened anteriorly, nearly bell-shaped or subcordiform, 1. 6 times as wide as long; the widest part of pronotum at its anterior fourth; anterior pronotal margin slightly but widely incurved, posterior margin slightly bisinuous; lateral pronotal margins widely arched in anterior half, nearly straight in posterior half, finely incurved anteriorly of hind corners which are rather sharp; sculpture of pronotum homogenous, consisting of small and dense, polygonal punctures (Fig. 2). Scutellum not preserved.

Elytra caudiform (Figs 1, 2), 2.4 times as long as wide, the widest part of elytra at their posterior third; humeral swellings not distinct; apex of each elytron narrowly incurved with small inner and outer teeth; elytra with fine and narrow longitudinal striae, interstices with shallow, rounded or slightly irregular punctures (well-preserved on right elytron) which are condensed into a distinct field at posterior third of lateral elytral margin; elytral suture seems to be slightly elevated. Hind wings surprisingly well-preserved but only their distal part is visible, the rest with the most important veins covered by elytra.

Last abdominal segment rather well-preserved, unfortunately mostly covered by left elytron; its posterior margin widely incurved with sharp lateral spines. Legs relatively long, anterior and middle tibiae straight; metatibiae slightly bent outwards and slightly widened at the midlength of inner margin.

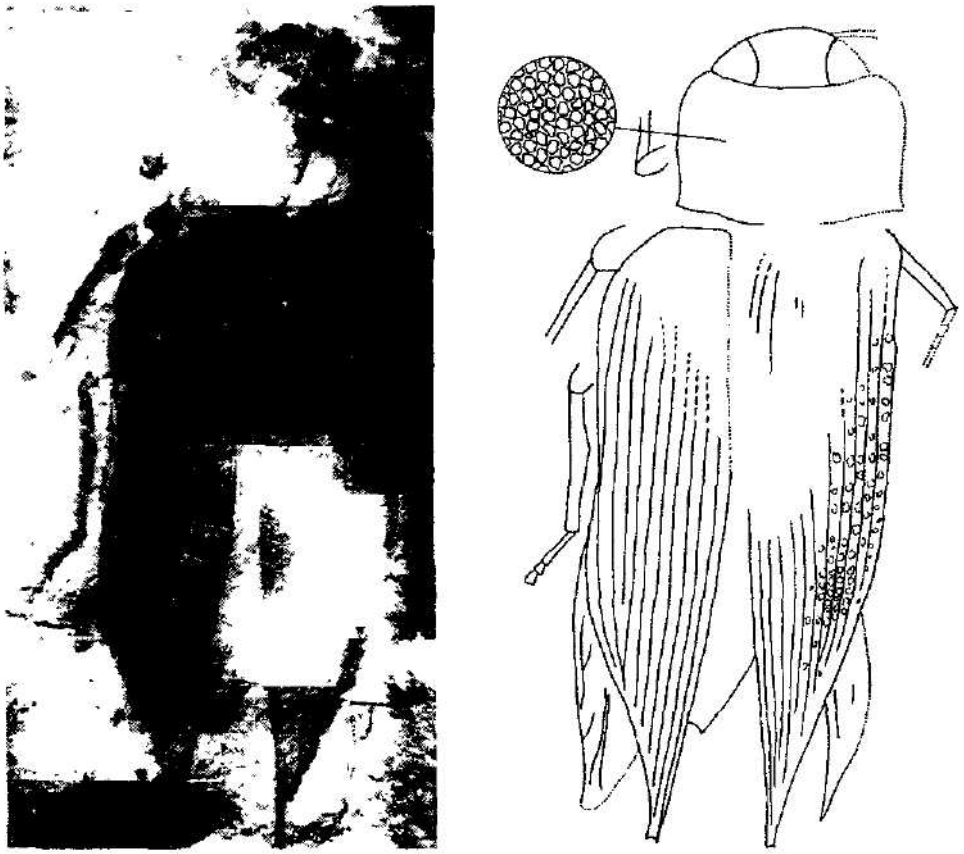
Measurements: expected length: 19.5 mm; length of head: 2.0 mm; width of head: 4.0 mm; length of thorax: 4.0 mm; width of thorax: 5.5 mm; length of elytra: 14.5 mm; width of elytra: 4.1 mm.

TYPE MATERIAL. **Holotype** (female): specimen ZD9704, North Bohemia, Bílina brown coal mine, 1992, Z. Dvořák leg.; type strata: Miocene (Eggenburgian/Ottnangian), Most Formation, Delta Sandy Horizon, in brown claystone Holotype deposited in the Bílina mine collection, Czech Republic.

NAME DERIVATION. *D. bilinica* sp. n. is named after the Bílina town, locality of the holotype.

DISCUSSION. Holotype of *D. bilinica* sp. n. is one of the best-preserved fossil beetle from tertiary claystone and there is easily possible to put it to the recent genus *Dicerca* and to the subgenus *Dicerca* s. str. After having compared it with nearly all recent species of this genus we found it to be very similar and close to *D. aenea* (Linnaeus, 1761) which is recently widely distributed in lowland forests throughout the whole Southern and Central Europe (host plant: *Populus* spp.). According to the form of tibiae (mesotibiae simple, metatibiae only with small, obtuse spine) and anal segment with short lateral spines we were able to determine also the sex – a female.

D. bilinica sp. n. differs from the recent species, *D. aenea*, by dense, homogenous pronotal sculpture without smooth fields; that of *D. aenea* is sparser, not homogenous with small, smooth fields on the disc of pronotum. Also apical parts of elytra are somewhat more caudiform than that of *D. aenea* (this character could be distorted in the course of fossilization).



Figs 1–2. *Dicerca* (s. str.) *bilinica* sp. n., holotype specimen ZD9704, 19.0 mm. 1 – photo; 2 – line drawing.

There are only a few fossil *Dicerca*-species described from Europe and North America: *D. reticulata* Assmann, 1870, *D. bronni* Heyden, 1859, *D. taschei* Heyden, 1856, all from Upper Oligocene, and *D. prisca* Heer, 1847 from Upper Miocene and one species D. eurydice Wickham, 1914 described from Oligocene locality Florissant in Colorado. According to the descriptions and illustrations of the species mentioned above (Assmann 1870, Heer 1847, Heyden 1856, 1859, Wickham 1914) they were described only after poor fragments and there is not sure if they really belong to the genus *Dicerca*, so there is impossible to compare them with *D. bilinica* sp. n. (to say nothing about the time-gap between them).

Next six species were described by Hörnschemeyer & Wedmann (1994) and Wedmann & Hörnschemeyer (1994) from Middle Eocene quarry Messel near Darmstadt (under *Dicerca* MeI 2138, MeI 1079, MeI 2734, MeI 1461, MeI 2289 and MeI 1190). Thanks to the detailed descriptions of these specimens (most of them possess also fairly well-preserved colours), illustration of MeI 2138 and colour photograph of the specimen MeI 2528, we are inclined to suppose them (according to their elytral and pronotal sculpture) to be more likely *Psiloptera* sp. or *Palmar* sp. than *Dicerca.*

Weidlich (1987) who studied fossil buprestid-beetles did not mention any *Dicerca*-species from Middle Eocene near Geiseltal.

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Beetle communities (Insecta: Coleoptera) of rock debris on the Boreč hill (Czech Republic: České středohoří mts)

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Abstract. Sixty seven beetle species (Coleoptera) in 3824 specimens were collected in rock debris of the Boreč hill (České středohoří mts, northern Bohemia, Czech Republic), in an all-year-round study in 1993–1994. Seven baited pitfall traps on the bottom margin (2), middle (2) and upper margin (3) of rock debris were serviced monthly. The majority of specimens was collected during vegetation season, with the maximum in October; the minimum of specimens was found in February. Comparison of monthly dominance is given. Using a WPGMA cluster analysis based on the Morisita's index of similarity, three main clusters are defined: 1. November, clustered separately from all other months of the year; 2. late spring-summer months (May–August) and 3. early spring (March–April) together with early autumnal (September–October) months. The Brillouin index of diversity decreases from the bottom to the upper margin of the rock debris, although the numbers of species are not significantly decreasing. Using the same procedure, samples from the central part of the rock debris clearly differ from those from marginal parts (bottom, upper margin). Seasonal abundance and spatial distribution of nine most abundant species is given for the following species (ordered from the most to the last abundant): *Catops picipes* (Fabricius, 1792), *Sciodrepoides watsoni watsoni* (Spence, 1815), *Choleva lederiana lederiana* Reitter, 1901, *Catops tristis tristis* (Panzer, 1794), *Catops subfuscus subfuscus* Kellner, 1846, *Bembidion stephensii* Crotch, 1866, *Pterostichus negligens* (Sturm, 1824), *Oxypoda vittata* Märkel, 1842 and *Omalium excavatum* Stephens, 1834.

Ecology, rock debris, seasonal abundance, spatial distribution, Coleoptera, Bohemia

INTRODUCTION

An exceptional type among Central European ecosystems, usually only minimally affected by human activities, is created by rock debris. Moreover, rock debris sometimes forms sites never covered by forests continually from the last glacial period (Růžička 1993). Usually, a specific microclimate is formed, suitable for many specific plant species with relict distribution (Pilous 1938, 1959, Kolbek 1983, Pujmanová 1988, 1989, 1990, Sádlo & Kolbek 1994) as well as invertebrates (Ložek 1954, Růžička 1988, 1989, 1990, 1994b, 1996b, Růžička et al. 1989, Christian 1993, Růžička & Zacharda 1994, Růžička et al. 1995, Čeřovský & Holec 1996).

The beetles inhabiting rock debris ecosystems were studied mostly in mountains (Obenberger 1952, Hůrka 1958, Martiš 1975, Molenda 1989, Růžička & Zacharda 1994), beetle communities of these ecosystems at lower altitudes are only poorly known (Růžička et al. 1989, Růžička 1996a).

The Boreč hill (449 m a. s. l.) is a part of the Kostomlatské středohoří mts, a western part of the České středohoří mts. The locality established as a national reserve in 1951 (Maršáková-Němejcová & Mihálik 1977) is well-known for the long time (Krejčí 1881, Šimr 1957, Kubát 1971) by its characteristic microclimatic features – the warm and wet exhalation from the top part cracks during colder winter months (in opportune conditions visible as white evaporation – Drahoš 1957); as well as the cold air exhalation and the presence of ice to late spring in the rock debris on the northern to northeastern foot (Pilous 1959, Šimr 1964, Jíra 1966, Kubát 1971). This phenomenon is probably more

complex than previously known, caused by crack connections reaching deep into the laccolith body of the hill (Váně 1992)

The locality has been the subject of several biological studies. Bryophytes were studied by Pilous (1959) and Pujmanová (1990), and molluscs were investigated by Ankert (1917) and Ložek (1954) A brief list of beetles was given by Novotny & Novotný (1966), the occurrence of two remarkable carabid beetles [*Leistus montanus* Stephens, 1827 and *Pterostichus negligens* (Sturm, 1824)] was recorded by Kubát (1971)

The aim of this study is to examine (1) the composition of beetle communities in rock debris and (2) the changes in the seasonal as well as spatial pattern of such communities

AREA OF STUDY

The beetles were collected in the rock debris on the north-eastern slope of the Boreč hill (50°31'N 14°00'E), about 0 5 km south-west of the Režny Ujezd village, mapping square code 5449d (see Pruner & Míka 1996), at 350 m a s l The free area of the rock debris is about 130 m wide and 20 to 25 m long, with 25–35° declination The rock debris is composed from flat stones (consisting from sodalitic trachyte), with the maximum diameter only a few centimetres on the upper margin and in several drifts in middle parts (Fig 18), usually 10 to 30 cm in the middle and on the bottom margin The bottom margin continually passes from the debris to a deciduous forest (with dominant *Sorbus*, *Betula*, *Quercus* and *Corylus* spp), on the left side changed by a small, wet, unimproved pasture Upper margin of the rock debris joins with a grassy slope with irregularly dispersed shrubs and individual *Pinus Betula* and *Sorbus* trees, and again passes from the debris to a dry and warm grove covering the top parts of the hill

The traps are labelled as No 1 to No VII throughout the text The field numbers of individual traps (used on locality labels of the voucher specimens) are also given in square brackets In total, seven traps were placed on the following sites

(a) Two traps were situated on the bottom margin of the rock debris *Trap No I* [field number 4] was placed in the right part with an adjacent deciduous forest, ca 2 5 m above the margin of the free rock debris, and ca 30 cm deep between stones with the maximum size of 10–30 cm *Trap No II* [field number 10] was placed in the left part with an adjacent pasture, 10 cm deep under a big stone on the margin of the rock debris, and close to a fissure with cold exhalation

(b) Two traps were placed in the middle part of the rock debris *Trap No III* [field number 5] was situated in the right part of the rock debris, ca 6 m above the bottom margin of the rock debris, and ca 30 cm deep between stones with the maximum size of 10–30 cm *Trap No IV* [field number 6] was placed in the central part, ca 10 m above the bottom margin of the rock debris, in a tongue of larger, slowly scrolling stones, and ca 30 cm deep between stones with maximum size 10–20 cm

(c) Three traps were situated on the upper margin of the rock debris *Trap No V* [field number 7] was placed in a fine debris in the right part at the upper margin, 15 cm deep between stones with the maximum size of only 2–6 cm *Trap No VI* [field number 8] was placed ca 2 m above the central part of the upper margin in a small but deep crevice, 15 cm deep between small stones, and close to a fissure with cold exhalation *Trap No VII* [field number 9] was placed in the left part of the upper margin, 20 cm deep between stones with the maximum size of 3–15 cm

MATERIAL AND METHODS

The material was collected using pitfall traps with an outlet of 10 cm diameter, 9 cm deep The traps were filled with 1 1 solution of water and ethylene glycol and covered with a net of mesh size 2 cm and a tin roof Fish meat and ripened cheese were used as bait, placed in a smaller container (1 5 cm deep), and over-hung above the level of the preserve solution The material was placed into 75% ethanol, a small part of voucher specimens was dry mounted and deposited in the author's collection Some Staphylinidae are also deposited in collection of the Šarišske muzeum, Bardejov The beetles were identified by the following specialists Carabidae – Pavel Moravec, Ptinidae – Miroslav Mikat, Staphylinidae – Tomaš Jászay, Pavel Moravec (*Tachinus*), Lubomír Hromadka (*Philonthus*), Petr Štourač (*Quedius*), Matuš Kocian (*Mycetoporus*), Silphidae, Leiodidae, Dermestidae, Nitidulidae – Jan Růžička, Cryptophagidae – Miroslav Řeška, Scarabaeidae – David Král, Dasytidae – Karel Majer Species names are treated according to Jelínek (1993) A few remnants of specimens of Staphylinidae can be determined only as *Atheta* spp 1 to 4

The traps were exposed between April 22, 1993 and May 30, 1994; and serviced 13 times with approximately monthly intervals.

For the cluster analysis, the y = ln (x) transformation was applied to the measured data. The Morisita's index of similarity was selected as the suitable index for quantitative data, strictly recommended because it is not dependent on the sample size (Krebs 1989). Hierarchical cluster analysis in Q-mode (study of similarity between pairs of the rows in the input matrix, here months or traps, Sneath & Sokal 1973) was performed. A weighted pair-group method using arithmetic averages (WPGMA) was used; it is appropriate for quantitative data according to Sneath & Sokal (1973).

The Brillouin index was used to measure species diversity. Its adequacy for pitfall trap samples was justified by Krebs (1989).

The program NTSYS-pc 1.80 (Rohlf 1994) was used for the cluster analysis.

RESULTS

Altogether, 3824 adult specimens of 67 species of Coleoptera were trapped. The most abundant beetle family was Leiodidae with 2965 specimens in 17 species, followed by Staphylinidae (384 specimens in 29 species), Carabidae (327 specimens in only 3 species) and Silphidae (112 specimens in 6 species), and only 36 specimens belonged to other 6 families (Tables 1, 2).

Seasonal abundance

During the year, samples considerably fluctuated in total abundance of beetles (Fig. 1). During the vegetation season (March–October), there were higher numbers of specimens with the maximum in October. In winter months (December–February), the total numbers decreased with the minimum in February. The number of species exhibited similar pattern (Fig. 2). The Brillouin index of diversity reached the maximum in spring months (April–May, 1994), with the minimum in November (Fig. 2). The abundance of individual species during the year is treated in Table 1.

The monthly dominance structures (Fig. 16) can be characterised as follows:

January 1994: Three species represented almost 70% of the species community – *Catops tristis tristis* (28.6%), *Oxypoda vittata* (25%) and *Choleva lederiana lederiana* (17.9%).

February 1994: Species community was very poor, composed only of 7 species represented by 9 specimens; only *Catops tristis tristis* was present in more than a single specimen.

March 1994: The species community was dominated by *Oxypoda vittata* (45.7%), next were *Catops tristis tristis* (22.4%), *Choleva lederiana lederiana* (11.2%) and *Catops longulus* (6%).

April 1994: Four species of Leiodidae represented almost 70% of the species community: *Catops subfuscus subfuscus* (19.7%), *C. tristis tristis* (16.5%), *Choleva lederiana lederiana* (16%) and *Sciodrepoides watsoni watsoni* (16%). *Omalium excavatum* (10.1%) reached the maximal year abundance.

May 1994: Five species of Leiodidae represented almost 75% of the species community: *Sciodrepoides watsoni watsoni* (31.4%), *Choleva lederiana lederiana* (20.3%), *Catops picipes* (10.9%), *C. tristis tristis* (6.2%), *C. subfuscus subfuscus* (5.3%) and *Omalium excavatum* (5.8%).

May 1993: Similarly, the same 5 species of Leiodidae represented more than 70% of the species community: *Catops subfuscus subfuscus* (26.3%), *Choleva lederiana lederiana* (19.7%), *Sciodrepoides watsoni watsoni* (9.9%), *Catops tristis tristis* (9.5%) and *C. picipes* (6.8%). Almost 20% of the species community was composed in this period also by two species of Carabidae: *Bembidion stephensii* (14.1%) and *Pterostichus negligens* (5.6%).

June 1993: Six species of Leiodidae and Carabidae represented 90% of the species community: *Sciodrepoides watsoni watsoni* (19.8%), *Catops picipes* (17.6%), *Pterostichus negligens* (17.4%), *Bembidion stephensii* (17.1%), *Catops subfuscus subfuscus* (10.2%) and *Choleva lederiana lederiana* (8.1%).

Tab 1 The summary abundance of individual species, monthly, the beetle communities of the rock debris on the Boreč hill, May 1993–May 1994

month	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	total	total
species	93	93	93	93	93	93	93	93	94	94	94	94	94		(%)
Catops picipes	35	74	47	187	152	188	24	0	0	0	0	0	49	756	19 77
Sciodrepoides w watsoni	51	83	244	115	10	3	0	0	0	0	0	60	141	707	18 49
Choleva l lederiana	102	34	32	11	17	121	46	5	5	1	13	60	91	538	14 07
Catops tristis tristis	49	18	10	41	46	186	2	3	8	3	26	62	28	482	12 60
Catops s subfuscus	136	43	8	3	0	1	0	0	0	0	1	74	24	290	7 58
Bembidion stephensii	73	72	15	0	1	9	0	3	0	0	1	2	1	177	4 63
Pterostichus negligens	29	73	17	13	10	2	0	0	0	0	0	1	3	148	3 87
Oxypoda vittata	1	0	0	0	0	21	3	4	7	1	53	15	1	106	2 77
Omalium excavatum	8	5	1	1	2	1	0	0	0	0	4	38	26	86	2 25
Catops longulus	3	9	5	1	8	17	0	0	3	1	7	10	10	74	1 94
Atheta crassicornis	1	0	13	10	8	2	0	0	0	0	1	15	11	61	1 60
Catops f fuliginosus	3	1	1	0	1	40	1	0	1	1	2	3	1	55	1 44
Aleochara curtula	6	0	0	23	1	0	0	0	0	0	0	0	6	36	0 94
Nicrophorus f fossor	0	0	16	13	2	0	0	0	0	0	0	0	0	31	0 81
Nicrophorus vespilloides	2	0	1	11	9	0	0	0	0	0	0	0	5	28	0 73
Nicrophorus humator	2	0	0	0	2	0	0	0	0	0	2	3	8	17	0 44
Silpha carinata	1	3	2	2	8	0	0	0	0	0	0	0	1	17	0 44
Cryptophagus pallidus	0	0	0	0	16	0	0	0	0	0	0	0	0	16	0 42
Nicrophorus vespillo	1	1	0	1	0	1	0	0	0	0	0	2	10	16	0 42
Catops westi	0	0	0	0	0	0	0	0	0	0	0	6	9	15	0 39
Omalium caesum	5	0	0	1	1	1	0	2	0	0	0	4	0	14	0 37
Catops grandicollis	0	0	0	0	9	3	0	0	0	0	0	0	0	12	0 31
Atheta triangulum	0	0	0	0	0	0	4	4	1	1	0	0	0	10	0 26
Proteinus atomarius	0	0	3	1	3	0	0	0	0	0	0	1	1	9	0 24
Catops c coracinus	0	1	1	2	0	0	0	0	0	0	0	2	2	8	0 21
Catops nigricans	0	0	0	0	2	5	1	0	0	0	0	0	0	8	0 21
Tachinus rufipennis	0	0	0	1	0	0	0	0	0	0	2	4	1	8	0 21
Cryptophagus pilosus	0	0	0	1	2	3	0	0	0	0	0	0	1	7	0 18
Choleva cisteloides	0	0	0	0	0	0	0	4	2	1	0	0	0	7	0 18
Atheta trinotata	1	0	0	1	0	0	0	0	0	0	0	4	1	7	0 18
Zyras humeralis	1	0	0	0	0	0	0	0	0	0	0	1	4	6	0 16
Atheta sodalis	1	0	0	0	1	0	0	0	0	0	0	1	2	5	0 13
Quedius mesomelinus	0	0	0	1	0	1	0	0	0	0	0	2	1	5	0 13
Catops chrysomeloides	1	0	0	0	0	3	0	0	0	0	0	0	0	4	0 10
Proteinus brachypterus	0	0	0	0	2	2	0	0	0	0	0	0	0	4	0 10
Danacea pallipes	0	1	0	0	1	0	0	0	0	0	0	1	0	3	0 08
Choleva o oblonga	0	0	0	1	0	1	0	0	0	0	1	0	0	3	0 08
Ptomaphagus sericatus	1	0	2	0	0	0	0	0	0	0	0	0	0	3	0 08
Olceoptoma thoracica	0	0	0	0	0	0	0	0	0	0	0	0	3	3	0 08
Atheta britanniae	0	0	0	1	0	1	0	0	0	0	0	0	1	3	0 08
Atheta europaea	1	0	0	0	0	0	0	2	0	0	0	0	0	3	0 08
Mycetoporus bosnicus	1	0	0	0	0	1	0	0	0	0	1	0	0	3	0 08
Omalium rivulare	0	0	0	0	0	1	0	0	1	0	0	0	1	3	0 08
Abax parallelepipedus	0	0	2	0	0	0	0	0	0	0	0	0	0	2	0 05
Nargus anisotomoides	0	0	0	0	0	1	0	0	0	0	0	0	1	2	0 05
Ptinus schlereth	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0 05
Aleochara stichai	0	0	0	0	0	1	0	0	0	0	1	0	0	2	0 05
Liogluta granigera	0	0	0	0	0	0	0	0	0	0	0	1	1	2	0 05
Philontus succicola	0	0	1	1	0	0	0	0	0	0	0	0	0	2	0 05
Cryptophagus acutangulus	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0 03
Cryptophagus distinguendus	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0 03
Cryptophagus nitidulus	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0 03

Tab. 1. Continued

month species	May 93	Jun 93	Jul 93	Aug 93	Sep 93	Oct 93	Nov 93	Dec 93	Jan 94	Feb 94	Mar 94	Apr 94	May 94	total	total (%)
Dermestes murinus	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0 03
Ptomaphagus varicornis	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0 03
Omosita discoidea	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.03
Ptinus pilosus	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0 03
Epauloecus unicolor	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.03
Onthophagus joannae	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0.03
Aleochara bipustulata	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0 03
Aleochara sparsa	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0.03
Atheta brevicollis	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0 03
Atheta marcida	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0 03
Atheta sp 1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0.03
Atheta sp. 2	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0 03
Atheta sp 3	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0 03
Atheta sp 4	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0 03
Stenus cf. *cicindeloides*	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0 03
total number of specimens	518	420	423	445	316	618	81	27	28	9	116	375	448	3824	100 00

July 1993: Three species of Leiodidae represented more than 75% of the species community: *Sciodrepoides watsoni watsoni* was dominant (57.7%), followed by *Catops picipes* (11.1%) and *Choleva lederiana lederiana* (7.6%).

August 1993: Again, 3 species of Leiodidae represented more than 75% of the species community: *Catops picipes* was dominant (42%), followed by *Sciodrepoides watsoni watsoni* (25.8%) and *Catops tristis tristis* (9.2%). *Aleochara curtula* (5.1%) reached the maximal year abundance.

September 1993: Three species of Leiodidae represented almost 70% of the species community: *Catops picipes* was again dominant (48.1%), followed by *C. tristis tristis* (14.6%) and *Choleva lederiana lederiana* (5.4%). Only this month, *Cryptophagus pallidus* (5%) was present.

October 1993: Four species of Leiodidae represented more than 85% of the species community: *Catops picipes* (30.4%) and *C. tristis tristis* (30.1%) were most abundant, followed by *Choleva lederiana lederiana* (19.6%) and *Catops fuliginosus fuliginosus* (6.5%). This month, the maximum of the total number of specimens was reached.

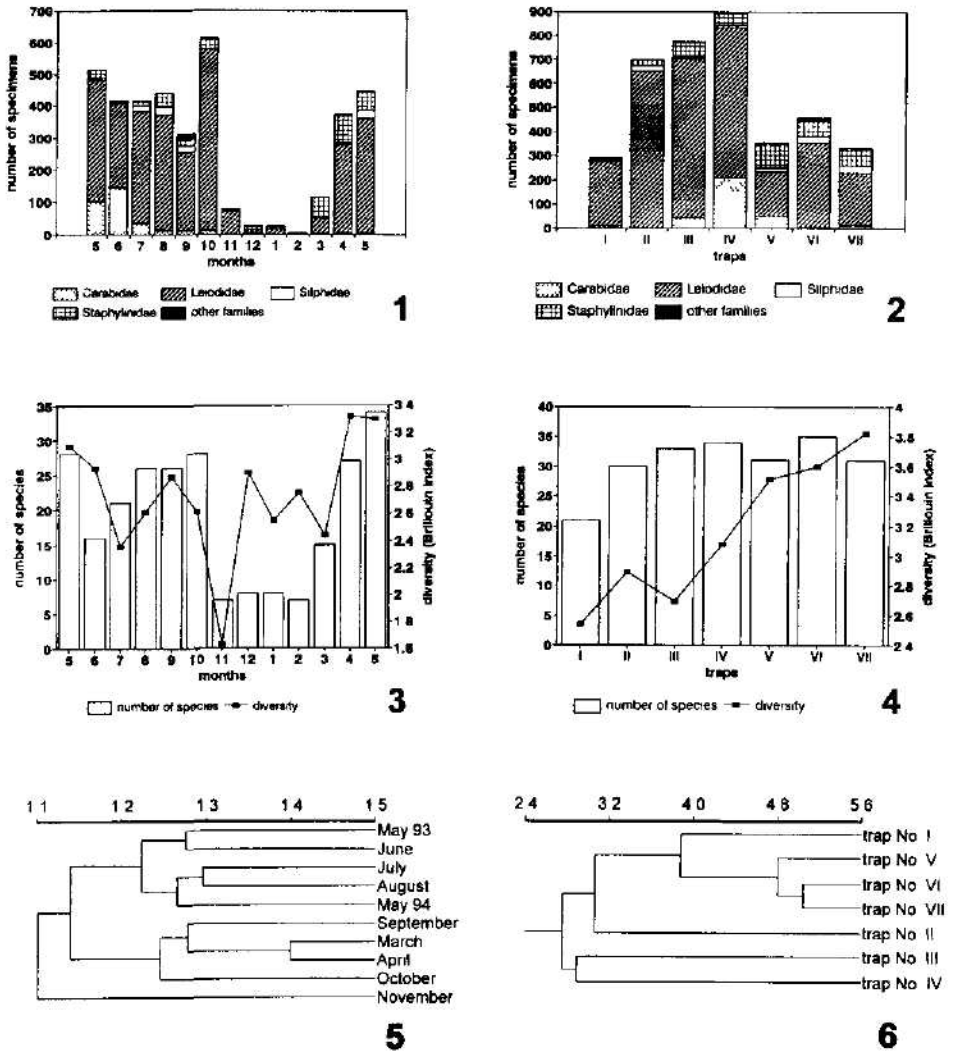
November 1993: Two species of Leiodidae represented more than 85% of the species community: *Choleva lederiana lederiana* was dominant (56.8%), followed by *Catops picipes* (29.6%).

December 1993: Four species of Leiodidae (2 species) and Staphylinidae (2 species) represented almost 65% of the species community: *Choleva lederiana lederiana* (18.5%), *C. cisteloides*, *Atheta triangulum* and *Oxypoda vittata* (all 14.8%).

Using a WPGMA cluster analysis based on the Morisita's index of similarity and ln-transformed data, three main clusters can be defined (Fig. 5): (1) November, which is clustered separately from all other months of the year; (2) late spring to summer months (May–August) and (3) early spring (March–April) and early autumn (September–October) months. The December–February samples were excluded from the analysis because of the low specimens numbers (see Table 1).

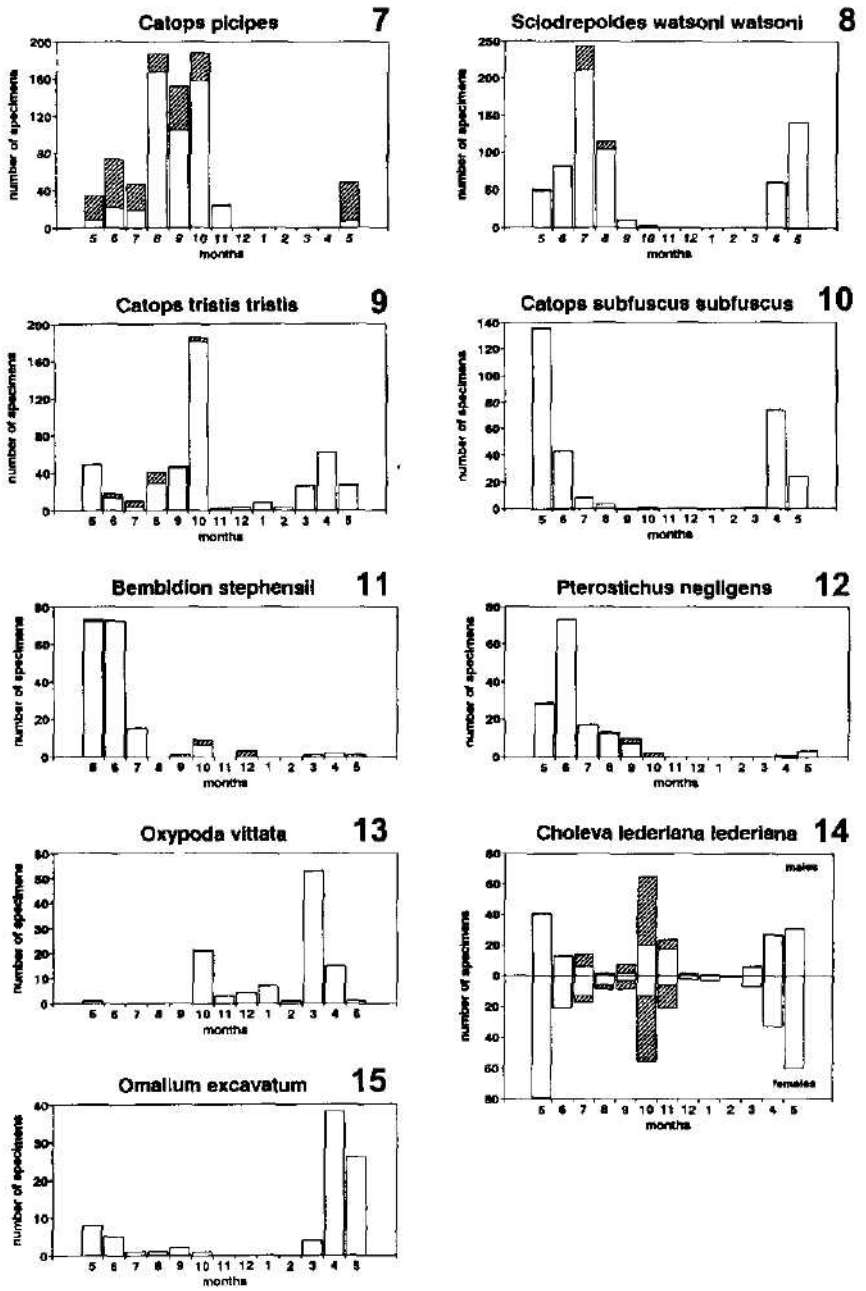
Trap preference

Individual traps situated in different positions in the rock debris differ in total abundances of beetles (Fig. 2). Most specimens were captured in the left bottom margin of the rock debris (trap No. II) adjacent to the pasture and in the central part of the rock debris (traps Nos. III and IV). The



Figs 1–6 Beetle community of the rock debris in the Boreč hill, May 1993 – May 1994 1 – total abundance of beetle families, monthly; 2 – total abundance of beetle families, individual traps, 3 – total number of species and the Brillouin index of diversity, monthly; 4 – total number of species and the Brillouin index, individual traps, 5 – cluster analysis (the Morisita's index of similarity, WPGMA, December–February samples excluded, see text for details), monthly, 6 – cluster analysis (the Morisita's index of similarity, WPGMA), individual traps

minimum of specimens as well as the lowest number of species was trapped in the right bottom margin of the rock debris with adjacent deciduous forest (trap No. I, Figs 2, 4). The Brillouin index of diversity decreases from the bottom to the upper margin (Fig. 4; Spearman rank correlation coefficient $r_s = 0.964$, $n = 7$, $p < 0.01$) although the numbers of species are not decreasing significantly (Fig. 4; $r_s = 0.036$, $n = 7$, n. s.). The abundance of individual species in traps is given in Table 2.



s 7–15 Seasonal abundance of species in the beetle community of the rock debris on the Boreč hill, May 1993 lay 1994. 7 – *Catops picipes*, 8 – *Sciodrepoides watsoni watsoni*, 9 – *Catops tristis tristis*, 10 – *C. subfuscus fuscus*, 11 – *Bembidion stephensii*, 12 – *Pterostichus negligens*, 13 – *Oxypoda vittata*, 14 – *Choleva lederiana eriana*, 15 – *Omalium excavatum*. Hatching indicates percentage of teneral specimens.

Tab 2 The summary abundance of individual species in traps, the beetle communities of the rock debris on the Boreč hill, May 1993–May 1994

trap No species	family	I	II	III	IV	V	VI	VII	total	total (%)
Catops picipes (Fabricius, 1792)	Leiodidae	69	154	356	33	50	79	15	756	19 77
Sciodrepoides w watsoni (Spence, 1815)	Leiodidae	11	81	118	260	69	108	60	707	18 49
Choleva l lederiana Reitter, 1901	Leiodidae	121	103	59	161	2	65	27	538	14 07
Catops tristis tristis (Panzer, 1794)	Leiodidae	48	221	13	43	39	49	69	482	12 60
Catops s subfuscus Kellner, 1846	Leiodidae	7	49	96	104	5	6	23	290	7 58
Bembidion stephensii Crotch, 1866	Carabidae	0	0	38	94	45	0	0	177	4 63
Pterostichus negligens (Sturm, 1824)	Carabidae	10	0	6	115	3	2	12	148	3 87
Oxypoda vittata Markel, 1842	Staphylinidae	0	1	1	2	65	24	13	106	2 77
Omalium excavatum Stephens, 1834	Staphylinidae	0	2	41	32	6	5	0	86	2 25
Catops longulus Kellner, 1846	Leiodidae	5	19	10	14	8	11	7	74	1 94
Atheta crassicornis (Fabricius, 1792)	Staphylinidae	0	1	2	6	13	12	27	61	1 60
Catops f fuliginosus Erichson, 1837	Leiodidae	3	12	3	2	8	18	9	55	1 44
Aleochara curtula (Goeze, 1777)	Staphylinidae	0	13	6	0	3	2	12	36	0 94
Nicrophorus f fossor Erichson, 1837	Silphidae	0	1	4	2	0	19	5	31	0 81
Nicrophorus vespilloides Herbst, 1784	Silphidae	1	5	2	0	2	5	13	28	0 73
Nicrophorus humator Olivier, 1790	Silphidae	0	0	2	3	4	0	8	17	0 44
Silpha carinata Herbst, 1783	Silphidae	0	15	0	1	0	1	0	17	0 44
Cryptophagus pallidus Sturm, 1845	Cryptophagidae	0	0	2	2	0	12	0	16	0 42
Nicrophorus vespillo (Linneaus, 1758)	Silphidae	0	0	1	3	8	1	3	16	0 42
Catops westi Krogerus, 1931	Leiodidae	5	2	1	1	2	1	3	15	0 39
Omalium caesum Gravenhorst, 1806	Staphylinidae	2	1	0	0	1	9	1	14	0 37
Catops grandicollis Erichson, 1837	Leiodidae	0	1	0	0	0	6	5	12	0 31
Atheta triangulum (Kraatz, 1856)	Staphylinidae	0	1	0	0	6	0	3	10	0 26
Proteinus atomarius Erichson, 1840	Staphylinidae	0	1	0	1	0	2	5	9	0 24
Catops c coracinus Kellner, 1846	Leiodidae	1	3	0	1	0	2	1	8	0 21
Catops nigricans (Spence, 1815)	Leiodidae	0	4	1	1	0	2	0	8	0 21
Tachinus rufipennis Gyllenhal, 1810	Staphylinidae	1	0	4	1	0	2	0	8	0 21
Cryptophagus pilosus Gyllenhal, 1828	Cryptophagidae	0	2	0	1	3	1	0	7	0 18
Choleva cisteloides (Frolich, 1799)	Leiodidae	0	2	0	3	1	1	0	7	0 18
Atheta trinotata (Kraatz, 1856)	Staphylinidae	1	1	0	0	0	4	1	7	0 18
Zyras humeralis (Gravenhorst, 1802)	Staphylinidae	0	0	1	1	4	0	0	6	0 16
Atheta sodalis (Erichson, 1837)	Staphylinidae	3	0	1	0	0	0	1	5	0 13
Quedius mesomelinus (Marsham, 1802)	Staphylinidae	1	0	1	2	0	0	1	5	0 13
Catops chrysomeloides (Panzer, 1798)	Leiodidae	0	1	1	0	0	2	0	4	0 10
Proteinus brachypterus (Fabricius, 1792)	Staphylinidae	0	0	0	0	0	1	3	4	0 10
Danacea pallipes (Panzer, 1793)	Dasytidae	0	0	1	1	1	0	0	3	0 08
Choleva oblonga oblonga Latreille, 1807	Leiodidae	1	0	0	2	0	0	0	3	0 08
Ptomaphagus sericatus (Chaudoir, 1845)	Leiodidae	0	0	1	1	1	0	0	3	0 08
Oiceoptoma thoracica (Linneaus, 1758)	Silphidae	0	1	0	0	0	0	2	3	0 08
Atheta britanniae Bernhauer et Scheerpeltz, 1926	Staphylinidae	0	0	0	0	0	0	3	3	0 08
Atheta europaea Likovsky, 1984	Staphylinidae	0	0	0	0	0	2	1	3	0 08
Mycetoporus bosnicus Luze, 1901	Staphylinidae	0	1	2	0	0	0	0	3	0 08
Omalium rivulare (Paykull, 1789)	Staphylinidae	1	1	0	0	0	1	0	3	0 08
Abax parallelepipedus (Piller et Mitterpacher, 1783)	Carabidae	0	0	0	0	1	1	0	2	0 05
Nargus anisotomoides (Spence, 1815)	Leiodidae	1	0	1	0	0	0	0	2	0 05
Ptinus schlerethi Reitter, 1884	Ptinidae	0	0	1	1	0	0	0	2	0 05
Aleochara stichai Likovsky, 1965	Staphylinidae	0	0	0	0	1	1	0	2	0 05
Liogluta granigera (Kiesenwetter, 1850)	Staphylinidae	0	0	2	0	0	0	0	2	0 05
Philontus succicola C G Thomson, 1860	Staphylinidae	0	1	0	1	0	0	0	2	0 05
Cryptophagus acutangulus Gyllenhall, 1828	Cryptophagidae	0	0	0	1	0	0	0	1	0 03

Tab. 2 Continued

trap No species	family	I	II	III	IV	V	VI	VII	total	total (%)
Cryptophagus distinguendus Sturm, 1845	Cryptophagidae	0	0	1	0	0	0	0	1	0 03
Cryptophagus nitidulus Miller, 1858	Cryptophagidae	0	0	0	0	0	1	0	1	0 03
Dermestes murinus Linnaeus, 1758	Dermestidae	0	0	0	0	0	1	0	1	0 03
Ptomaphagus varicornis (Rosenhauer, 1847)	Leiodidae	0	1	0	0	0	0	0	1	0 03
Omosita discoidea (Fabricius, 1775)	Nitidulidae	0	0	0	1	0	0	0	1	0.03
Ptinus pilosus P. W. J. Müller, 1821	Ptinidae	0	0	0	0	0	0	1	1	0 03
Epauloecus unicolor (Piller et Mitterpacher, 1783)	Ptinidae	0	0	0	0	1	0	0	1	0.03
Onthophagus joannae Goljan, 1953	Scarabaeidae	0	0	0	0	1	0	0	1	0 03
Aleochara bipustulata (Linnaeus, 1761)	Staphylinidae	0	0	0	0	1	0	0	1	0 03
Aleochara sparsa Heer, 1839	Staphylinidae	0	0	0	0	1	0	0	1	0 03
Atheta brevicollis (Baudi, 1848)	Staphylinidae	0	0	0	0	1	0	0	1	0 03
Atheta marcida (Erichson, 1837)	Staphylinidae	0	0	0	0	0	0	1	1	0.03
Atheta sp 1	Staphylinidae	1	0	0	0	0	0	0	1	0.03
Atheta sp 2	Staphylinidae	0	0	0	0	0	0	1	1	0 03
Atheta sp 3	Staphylinidae	1	0	0	0	0	0	0	1	0 03
Atheta sp 4	Staphylinidae	0	0	1	0	0	0	0	1	0.03
Stenus cf *cicindeloides* (Schaller, 1783)	Staphylinidae	0	0	0	1	0	0	0	1	0 03
total number of specimens		294	701	780	898	356	459	336	3824	100 00

In the following, the dominance structure of individual traps (Fig. 17) is commented:

Trap No. I: Three species of Leiodidae represented 80% of the species community: *Choleva lederiana lederiana* was dominant (41.1%), followed by *Catops picipes* (23.5%) and *Catops tristis tristis* (16.3%).

Trap No. II: Five species represented almost 70% of the species community: *Catops tristis tristis* (31.5%, reached here the maximum of abundance compared with other traps), *C. picipes* (22%), *Choleva lederiana lederiana* (14.7%), *Sciodrepoides watsoni watsoni* (11.6%) and *Catops subfuscus subfuscus* (7%).

Trap No. III: Six species represented more than 90% of the species community: *Catops picipes* was dominant (45.6%), reaching here the maximum of abundance compared with other traps, followed by *Sciodrepoides watsoni watsoni* (15.1%), (12.3%), *Choleva lederiana lederiana* (7.6%), *Omalium excavatum* (5.3%) and *Bembidion stephensii* (4.9%).

Trap No. IV: Eight species represented almost 95% of the species community: *Sciodrepoides watsoni watsoni* (29%), *Choleva lederiana lederiana* (17.9%), *Pterostichus negligens* (12.8%), *Catops subfuscus subfuscus* (11.6%) and *Bembidion stephensii* (10.5%, reached the maximum of abundance here compared with other traps), followed by *Catops tristis tristis* (4.8%), *Catops picipes* (3.7%) and *Omalium excavatum* (3.6%).

Trap No. V: Five species represented 75% of the species community: *Sciodrepoides watsoni watsoni* (19.4%), *Oxypoda vittata* (18.3%, reached here the maximum of abundance compared with other traps), *Catops picipes* (14%), *Bembidion stephensii* (12.6%) and *Catops tristis tristis* (11%).

Trap No. VI: Five species represented more than 70% of the species community: *Sciodrepoides watsoni watsoni* (23.5%), *Catops picipes* (17.2%), *Choleva lederiana lederiana* (14.2%), *Catops tristis tristis* (10.7%) and *Oxypoda vittata* (5.2%).

Trap No. VII: Five species represented almost 60% of the species community: *Catops tristis tristis* (20.5%), *Sciodrepoides watsoni watsoni* (17.9%), *Choleva lederiana lederiana* (8%), *Atheta*

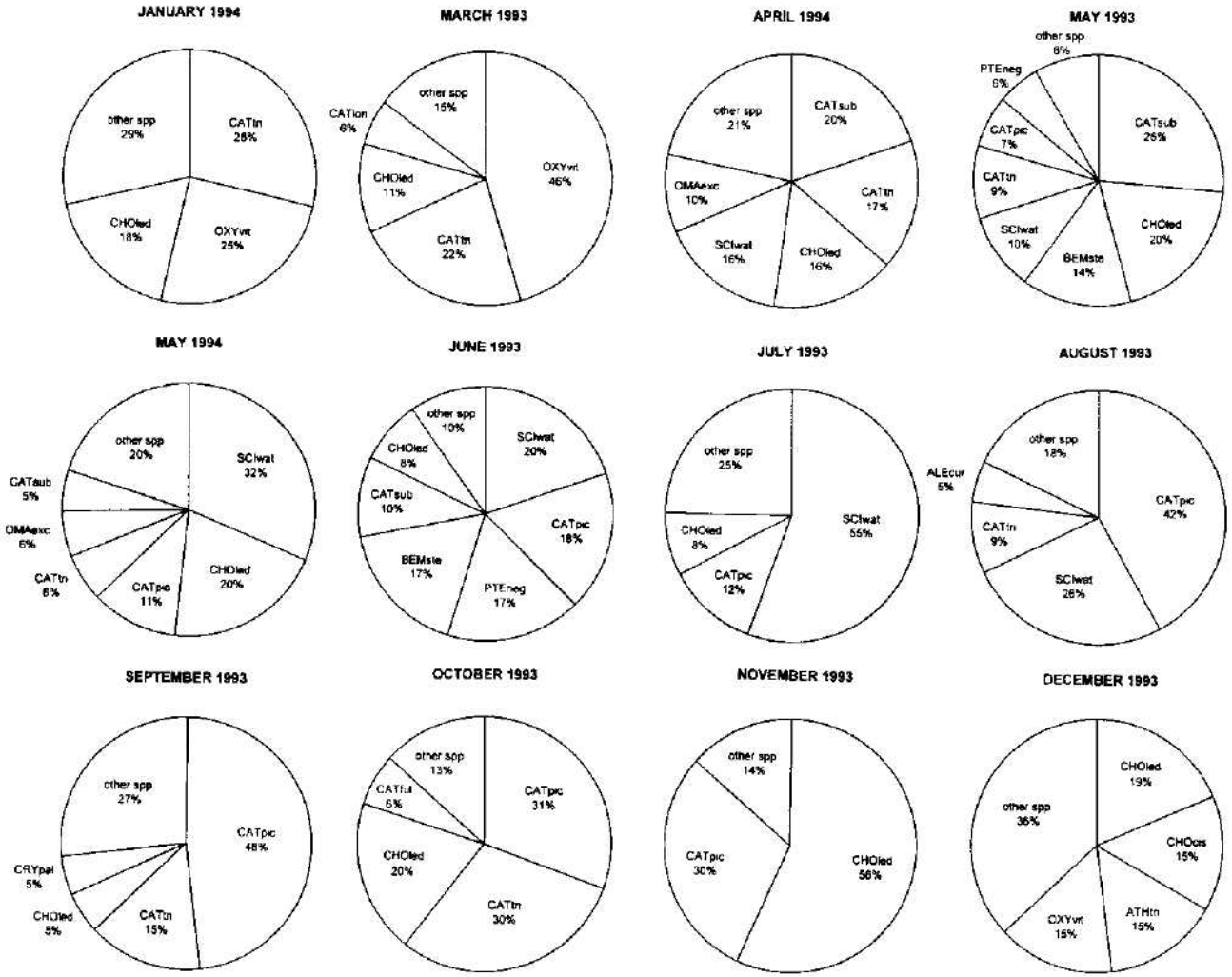

JANUARY 1994
other spp 29%
CATtn 28%
CHOled 18%
OXYvrt 25%
MARCH 1993
CATion 6%
other spp 15%
OXYvrt 46%
CHOled 11%
CATtn 22%
APRIL 1994
other spp 21%
CATsub 20%
OMAexc 10%
CATtn 17%
SCIwat 16%
CHOled 16%
MAY 1993
other spp 8%
PTEneg 6%
CATpic 7%
CATsub 25%
CATtn 9%
SCIwat 10%
CHOled 20%
BEMste 14%
MAY 1994
other spp 20%
SCIwat 32%
CATsub 5%
OMAexc 6%
CATtn 6%
CATpic 11%
CHOled 20%
JUNE 1993
other spp 10%
CHOled 8%
SCIwat 20%
CATsub 10%
CATpic 18%
BEMste 17%
PTEneg 17%
JULY 1993
other spp 25%
SCIwat 55%
CHOled 8%
CATpic 12%
AUGUST 1993
other spp 18%
ALEcur 5%
CATpic 42%
CATtn 9%
SCIwat 26%
SEPTEMBER 1993
other spp 27%
CATpic 48%
CRYpal 5%
CHOled 5%
CATtn 15%
OCTOBER 1993
other spp 13%
CATpic 31%
CATtn 6%
CHOled 20%
CATtn 30%
NOVEMBER 1993
other spp 14%
CATpic 30%
CHOled 56%
DECEMBER 1993
CHOled 19%
other spp 36%
CHOcs 15%
OXYvrt 15%
ATHtn 15%

Fig. 16 Dominance structures (relative abundances, May 1993 to May 1994, sample of February 1993 omitted) in the beetle community of the rock debris on the Borč hill, monthly. Abbreviations: ALEcur – *Aleochara curtula*, ATHtri – *Atheta triangulum*, BEMste – *Bembidion stephensii*, CATful – *Catops fuliginosus fuliginosus*, CATlon – *Catops longulus*, CATpic – *Catops picipes*, CATsub – *Catops subfuscus subfuscus*, CATtri – *Catops tristis tristis*, CHOcis – *Choleva cisteloides*, CHOled – *Choleva lederiana lederiana*, CRYpal – *Cryptophagus pallidus*, OMAexc – *Omalium excavatum*, OXYvit – *Oxypoda vittata*, PTEneg – *Pterostichus negligens*, SCIwat – *Sciodrepoides watsoni watsoni*

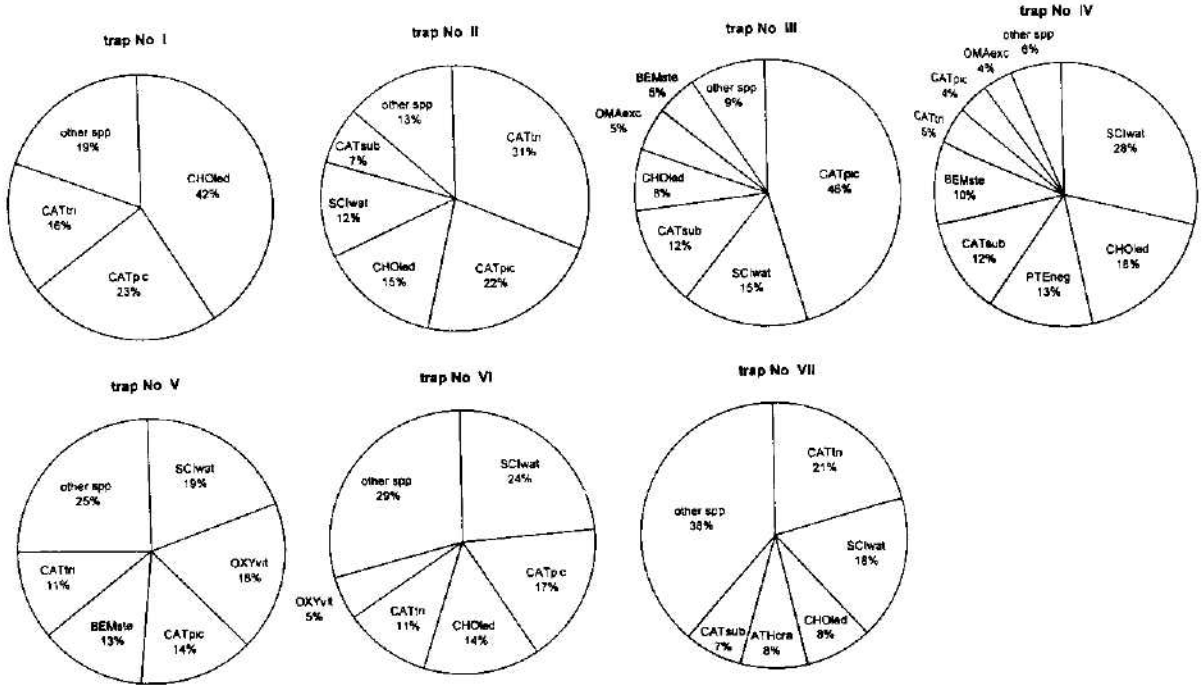


Fig. 17 Dominance structures (relative abundances) in the beetle community of the rock debris on the Borč hill, May 1993 – May 1994, individual traps. Abbreviations: ATHcra – *Atheta crassicornis*, BEMste – *Bembidion stephensii*, CATpic – *Catops picipes*, CATsub – *Catops subfuscus subfuscus*, CATtri – *Catops tristis tristis*, CHOled – *Choleva lederiana lederiana*, OMAexc – *Omalium excavatum*, OXYvit – *Oxypoda vittata*, PTEneg – *Pterostichus negligens*, SCIwat – *Sciodrepoides watsoni watsoni*

crassicornis (8%, reached here the maximum of abundance compared with other traps) and *Catops subfuscus subfuscus* (6.9%).

Using a WPGMA cluster analysis based on the Morisita's index of similarity and ln-transformed data, two main clusters of traps can be defined (Fig. 6): (1) traps from the central part (traps Nos. III and IV) and (2) traps from the marginal parts of the rock debris. Moreover, the traps from the upper margin (traps Nos. V–VII) are very closely branched in the second cluster, and further clustered with trap No. II and trap No. I from the bottom margin of the rock debris (Fig. 6).

Seasonal and spatial structure of abundance of frequent species

The nine most abundant species (each with at least 75 specimens, exceeding 2% of relative abundance) are sorted in the order of total abundance. In Figs 7–15, seasonal abundance of species (with percentage of teneral specimens indicated) is figured. The spatial abundance of species can be seen also in Table 2.

Catops picipes (Leiodidae): adult specimens were present from May to November, with a wide peak in August–October. The species breeds in late autumn, with preimaginal stages in winter months; teneral adults were present during almost the whole vegetation season (Růžička 1994; Fig. 7). In the traps, the species dominated in the middle part of the rock debris (namely in trap No. III) and also on the bottom margin, adjacent to the pasture (trap No. II).

Sciodrepoides watsoni watsoni (Leiodidae): adult specimens were present from April to October, with a single peak in July; teneral specimens were present mostly in July–August (Fig. 8). This species was most abundant in the middle part (traps Nos. III and IV) and also in the central part of the upper margin of the rock debris (trap No. VI).

Choleva lederiana lederiana (Leiodidae): the species was recently discovered in several cold rock debris ecosystems in northern Bohemia (J. Růžička & J. Vávra, unpubl.), and the data on the seasonal abundance of Central European populations are presented here for the first time and in a more detailed form than for other species, with male and female specimens figured separately. The adults were present during all months of the year, with two peaks in April–May and October–November, with teneral adults from July to November (Fig. 14). The pattern indicates bimodal adult activity with breeding in the late spring, and with over-wintering adults of the filial generation. The species was most abundant in the middle part (especially in trap No. IV) and in the bottom part of the rock debris (traps Nos. I and II).

Catops tristis tristis (Leiodidae): together with the previous species, adults were present during all months of the year, with a distinct peak in October (Fig. 9). The species was most abundant in the bottom part of the rock debris, adjacent to the pasture (trap No. II).

Catops subfuscus subfuscus (Leiodidae): adults were present from March to October, with a wide peak in April–June (Fig. 10). The species was most abundant in the middle part of the rock debris (traps Nos. III and IV).

Bembidion stephensii (Carabidae): adults were present from March to December, with a peak in May–June; teneral adults were present mostly in September–December (Fig. 11). The species was present in middle parts (traps Nos. III and IV) and in the upper part of the rock debris, between smaller stones (trap No. V).

Pterostichus negligens (Carabidae): adults were present from April to October, with a peak in May–June; teneral adults were present mostly in August–October, a few also in May (Fig. 12). The species was most abundant in the middle part of rock debris (especially, trap No. IV).

Oxypoda vittata (Staphylinidae): adults were present in October–May, more abundantly in October and March–April (Fig. 13). The species was most abundant in the upper part of the rock debris (traps Nos. V–VII).

Fig. 18. The general aspect of the rock debris, north-eastern slope of the Boreč hill, the České středohoří mts.

Omalium excavatum (Staphylinidae): adults were present from March to October, with peak in April–May (Fig. 15). The species was most abundant in the middle part of the rock debris (traps Nos. III and IV).

DISCUSSION

In this paper, an all-year-round seasonal abundance pattern of beetles in rock debris is presented for the first time. However, this pattern is generally similar to that found in the all-year-round study of coprophagous beetle community on pastures in south-western Germany (Waßmer 1994). Results differ slightly from those observed in western Germany in beetle communities from forested habitats, taken by unbaited pitfall traps (Köhler 1996: 199–200). Namely, main differences can be observed in the size of samples from the late fall (the highest number of specimens found in this study was achieved in October). These differences should be probably strongly influenced by the different microclimate of the rock debris ecosystems. The surface of the rock debris is not frozen at that time unlike the soil surface in open landscape (J. Růžička pers. obs.).

The fact that the Brillouin index of diversity has the decreasing tendency from bottom to upper margin of rock debris although the numbers of species are not significantly decreasing, is interesting. The differences observed should be influenced by various microclimatic conditions as well as by the proportions of stones along the vertical gradient of the rock debris (Růžička 1993). Similar

differences were found for several spider and mite species in rock debris ecosystems (Růžička et al 1995, Čeřovský & Holec 1996)

The seasonal abundance of most dominant species of Leiodidae is similar to the pattern observed by Růžička (1994a) in central Bohemia, and that of *Pterostichus negligens* corresponds to the results published by Martiš (1975) from the Krkonoše mts

The pattern of seasonal abundance of *Choleva lederiana lederiana* is similar to that described by Bistrom & Hippa (1987) from the Torhola cave in south-western Finland but differs strictly from the known pattern of *C lederiana holsatica* Benick et Ihssen in Benick, 1937 in Segeberger Hohle, a deep cave system in northern Germany, where the maximum peaks of adult activity occur in January–March and July–August, and the population reproduces mainly during winter months (Heun 1955, Zwick 1966) These differences can be related with the changes of food availability for this supposed generalist scavenger, with the possible maximum of food in rock debris or shallow caves during the vegetation season A reversed pattern can be observed in deep cave systems, with maximum of food supply (mainly guano) produced during winter by hibernating bats (Ipsen 1997)

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The life history of *Leishmania* (Kinetoplastida: Trypanosomatidae)

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Abstract This paper summarizes the current state of research concerning protozoan parasites of the genus *Leishmania* Ross, 1903 and its vectors, phlebotomine sandflies. The leishmanias, causative agents of leishmaniasis, a medically important disease, are an excelent example of a successful adaptation to parasitic life. In the present review, both parts of the *Leishmania* life-cycle, i. e. development of the parasite in the sandfly and the parasite-macrophage interaction in the vertebrate host, are analyzed. Special attention is given to factors influencing susceptibility of sandflies to *Leishmania*, and factors influencing *Leishmania* virulence. The role of lipophosphoglycan and gp63, two main cell-surface molecules, in parasite survival is discussed.

Leishmaniasis, sandfly, *Phlebotomus*, life-cycle, transmission, virulence, macrophage, lipophosphoglycan, gp63

INTRODUCTION

Leishmania spp. are causative agents of leishmaniasis, one of the six most important human parasitic diseases in the world. The medical importance of this genus stimulates medical and highly diversified basic biological research. Most frequent are biochemical, genetical and immunological studies (hundreds of papers published each year). Intensive research concerns virulence factors and drug targets. Less abundant, but equally important, is the research in the field of epidemiology, vector biology and vector-parasite interactions.

Whereas most recent reviews concentrate on particular problems, the present review attempts to inform on the main lines of the current leishmania research ranging from molecular biology to ecology. Equal attention is given to both the vector and host parts of the *Leishmania* life-cycle. Factors influencing *Leishmania* development in the vector, as well as *Leishmania* interactions with the defense system of host cells, are analyzed. The importance of main surface molecules of the *Leishmania*-cell during the life-cycle is discussed.

Leishmania is an excelent example of a successful adaptation to parasitic life. The intensive research on this parasite gives us the chance to recognize many details of the parasite – host – vector interactions.

PART I
Human leishmaniasis: a current status of the disease

Leishmaniasis is a disease caused by protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) transmitted by the bite of an insect vector, the phlebotomine sandfly (Diptera: Psychodidae). The life cycle of *Leishmania* includes morphologically and physiologically distinct forms in the sandfly and in the vertebrate host (Fig. 1). Parasites taken with a blood meal by female

sandflies transform into flagellated promastigote stages and undergo multiplication and differentiation from procyclic forms into infective, metacyclic forms in the sandfly gut. Metacyclic promastigotes are transmitted to the host during the next blood meal. In its tissue, they are phagocytosed by macrophages and, subsequently, survive and multiply in macrophage phagolysosomes as non-flagellated amastigote stages.

A number of *Leishmania* spp. cause disease in animals, and humans become infected incidentally when they enter an area of endemicity. Rarely humans serve as a reservoir. The clinical manifestation of the disease depends on complex interactions between the virulence characteristics of the parasite and the immune response of its host. Generally, the disease takes four main forms. **Visceral leishmaniasis (VL)** is the most serious form, fatal if untreated. **Cutaneous leishmaniasis (CL)** is the most common infection, causing simple or a limited number of skin lesions which usually heal in few months, but leave scars. **Mucocutaneous leishmaniasis (MCL)** begins with simple skin ulcers, which can spread and cause destruction of the nose, mouth, pharynx, or larynx tissues. **Diffuse cutaneous leishmaniasis (DCL)** is characterized by disseminated chronic lesions, difficult to treat. A single *Leishmania* species can produce more than one clinical syndrome and each syndrome is caused by multiple species (Pearson & de Queiroz Sousa 1996).

Leishmaniasis currently affects about 12 million people, the global annual incidence is estimated at 1–1.5 million new cases of CL and 0.5 million new cases of VL per year. These official data are strongly underestimated as they are obtained almost exclusively through a passive case detection. Leishmaniasis is endemic in 88 countries of all the continents except Australia and Antarctica, 350 million people being at the risk of infection. More than 90% of VL cases in the world are reported from Bangladesh, Brazil, India and Sudan and more than 90% of CL cases came from Afghanistan, Iran, Saudi Arabia, Syria, Brazil and Peru. MCL is an important problem in Brazil and Latin America. An outbreak of VL in Southern Sudan involved 100.000 deaths over the past five years in a population of less than one million. The importance of leishmaniasis increases today with expansion of AIDS: in southern Europe, high number of adult VL cases is related to HIV infection (data obtained from WHO web page; http://www.who.int/ctd/html).

PART II.
The systematic position and phylogeny of the genus *Leishmania*

Leishmania is a monophyletic genus (Thomaz-Soccol et al. 1993a) belonging to the family Trypanosomatidae (order Kinetoplastida). This ancient family involves species which are morphologically very similar, but their adaptations to many different life strategies led to a great heterogeneity in physiological and biochemical traits.

There are two opinions on the origin of digenetic trypanosomatids: 1. origin from monogenetic parasites of invertebrates, with subsequent adaptation to vertebrates, and 2. origin in vertebrates, with secondary transmission by blood-sucking invertebrates (reviewed by Lainson & Shaw 1987). The first hypothesis is supported mainly by (a) a common occurrence of monogenetic flagellates in various ancient invertebrate host groups, (b) hindgut development and contaminative transmission with the excreta in such parasites, (c) sexual reproduction of some trypanosomes in the insect vector, (d) more complicated development of trypanosomatids in invertebrates as compared to the development in vertebrates. The strongest argument for the second hypothesis is that monogenetic insect flagellates are more frequently found in invertebrate orders and families that feed on blood than in other taxa.

The origin of *Leishmania* from monogenetic insect flagellates is generally more widely accepted. According to this hypothesis, the origin of the genus *Leishmania* depends on the evolutional history of sandflies. Ancestors of modern sandflies probably existed as early as in the Jurassic era

and ancestors of Phlebotominae *sensu stricto* were found in Cretaceous deposits (Hennig 1972). From the Jurassic period, ancestral parasites might get established in vertebrate hosts. It is suggested that the haematophagous habit of sandflies was primarily associated with mammals, which is in agreement with the absence of *Leishmania* spp. in birds and their relative rarity in reptiles (Shaw 1997).

The *Leishmania* adaptation to mammals has run for about 90 million years, i. e., approximately, from the period when mammals were diversifying into different orders. When the African and South American continents divided, the subgenera *Leishmania* and *Viannia* became separated together with their hosts and vectors. Indeed, the genetic distance between the species of the New World subgenus *Viannia* and the subgenus *Leishmania* (Beverley et al. 1987) is of the same order as that of mammalian orders that separated some 85 million years ago (reviewed by Shaw 1997). The ancient division and the subsequent independent evolution of the two subgenera were confirmed also by a cladogram based on isoenzyme analysis (Thomaz-Soccol et al. 1993a). The subgenus *Leishmania* contains two sister groups of the Old World and the New World species complexes, respectively (Thomaz-Soccol et al. 1993a). The authors suggest that New World members of the subgenus *Leishmania* were introduced into American continent secondarily during early Cenozoic by migration of rodents from the Old World (Fig. 2). The subgenus *Sauroleishmania* containing reptile parasites diverged from the lineage leading to the subgenus *Leishmania* after this group separated from the *Viannia* subgenus (Noyes et al. 1998).

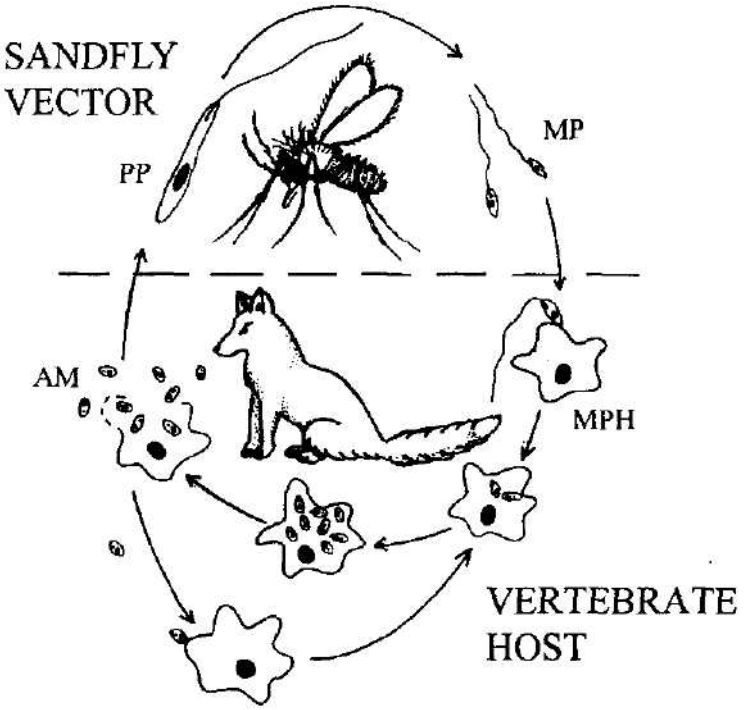

Fig. 1. Life-cycle of *Leishmania* parasites. PP, procyclic promastigotes; MP, metacyclic promastigotes; AM, amastigotes; MPH, macrophages.

The criteria used for the classification of leishmanial parasites have changed in time from primarily epidemiological, behavioral and clinical characters (influenced also by host and vector characters) to more objective methods of molecular biology, biochemistry, immunology, serology and ultrastructural morphology (reviewed by Schnur & Greenblatt 1995). After Shaw (1994) there are today 30 named species of *Leishmania* from mammals, 10 in the Old World and 20 in the New World (Tab. 1). Many of the recognized species share characters which allow to group them into species complexes (Lainson & Shaw 1987). The isoenzyme analysis of Thomas-Soccol et al. (1993a, b) support the classification of Lainson & Shaw (1987) and defines the phylogenetic hierarchy of the complexes (clusters of related zymodemes).

The classification of the *Leishmania*-like parasites of Old World reptiles is still controversial. Saf'janova (1982) originally used the term *Sauroleishmania* for the subgenus which groups the reptilian *Leishmania* spp. This classification is consistent with recent DNA-based evidence of a close relatedness of these parasites to the subgenus *Leishmania* (Noyes et al. 1998). However, the generic rank *Sauroleishmania* established by Killick-Kendrick et al. (1986) is still accepted (Lainson & Shaw 1989, Telford 1995).

PART III.
The taxonomy and biology of sandflies (Diptera: Phlebotominae)

Phlebotomine sandflies are small (1.5–3.5 mm) hairy flies characterized by erect narrow wings, slender bodies, long legs, black eyes and long mandibles (Fig. 3). There are about 700 recognized species grouped in several genera within the subfamily Phlebotominae of the family Psychodidae. Sandflies are widely distributed in tropics and other warm climate mainland areas. Northwards they extend to the latitude 48–50°, southwards to about 40°. Species in three genera, *Phlebotomus* Rondani, *Lutzomyia* França and *Sergentomyia* França et Parrot, suck blood from vertebrates. The former two genera are medically important as they contain disease vectors. The genus *Phlebotomus* occurs in the Old World, mostly in semiarid and savannah areas. *Lutzomyia* species are found only in New World tropics, where they prefer forested areas. *Sergentomyia* representatives are Old World sandflies feeding mainly on reptiles; some species bite humans but do not transmit *Leishma-*

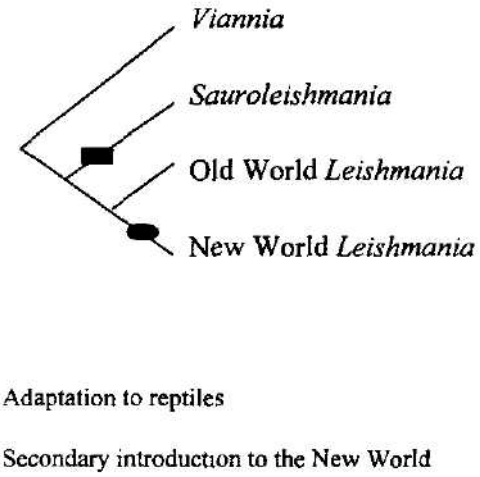

Fig 2 Phylogeny of the genus *Leishmania*.

Table 1 Classification and basic characteristics of named *Leishmania* species Based on Lainson & Shaw (1987), Lewis & Ward (1987), Killick-Kendrick (1990), Lawyer et al (1991), Lane (1993), Thomaz-Soccol et al (1993a, b), Shaw (1994), Jaramillo et al (1994), Schnur & Greenblat (1995), Telford (1995) and Strelkova (1996)

	Reservoir hosts[a]	Geographical distribution	Disease in man [b]	Vector species [c]
Genus *Leishmania* Ross, 1903 Subgenus *Sauroleishmania*[d] Ranque, 1973				
L agamae David, 1929	*Agama stellio*	Mediterranean and the Middle East		(*P papatasi*)
L ceramodactyli Adler et Theodor, 1929	*Ceramodactylus doriae*	Mediterranean and the Middle East		(*P papatasi*)
L tarentolae Wenyon, 1921	*Tarentola mauritanica* *T annularis* *Cyrtodactylus kotschyi*	Mediterranean and the Middle East		(*Se antennata*) (*P papatasi*)
L gymnodactyli Khodukin et Sofiev, 1940	*Crossobamon eversmanni* *Gymnodactylus caspius* *Agama sanguinolenta* *Eremias intermedia*	Southern Asia		(*Se arpaklensis*) (*P papatasi*)
L hemidactyli Mackie, Gupta et Swaminath, 1923	*Hemidactylus gleadowi*	Southern Asia		
L nicollei Khodukin et Sofiev, 1940	*Agama sanguinolenta* *Phrynocephalus helioscopus* *Ph mystaceus* *Ph interscapularis*	Southern Asia		
L gulikae Ovezmukhammedov et Saf'janova, 1987	*Agama caucasica*	Southern Asia		
L adleri Heisch, 1958	*Latastia longicaudata*	Africa		(*Se clydei*)
L hoogstraali McMillan, 1965	*Hemidactylus turcicus*	N Africa		
L senegalensis Ranque, 1973	*Tarentola annularis*	Africa		
Subgenus *Leishmania* Saf'janova, 1982 **Old World species complexes**				
***L. donovani* complex**				
L donovani (Laveran et Mesnil, 1903)	humans	E Pakistan, India, Nepal, China	VL, PKDL	***P. argentipes*** ***P. alexandri*** *P caucasicus*
L archibaldi Castellani et Chalmers, 1919	rodents, carnivores	Sudan, Ethiopia, Djibouti, Somalia, Chad, Niger, Congo, Central African Republic	VL, CL, OL	***P. martini***, ***P. orientalis*** *P celiae* *P vansomerenae*

***L. infantum* complex**				
L. infantum Nicolle, 1908	wild canids, (dogs)	Central and SW Asia, NW China, Middle East, Balkans, Mediterranean littoral, N and sub-Saharan Africa,	VL, CL, AL	***P. ariasi*** ***P. perniciosus*** ***P. perfiliewi*** ***P. neglectus*** ***P. chinensis*** ***P. langeroni*** *P. longiductus* *P. kandelaki* *P. longicuspis* *P. smirnovi* *P. tobbi* *P. transcaucasicus*
L. chagasi Cunha et Chagas, 1937*	wild canids, (dogs)	From Argentina to Mexico	VL, CL, AL	***Lu. longipalpis***
***L. tropica* complex**				
L. tropica (Wright, 1903)	?, (humans, dogs, rodents)	Mediterranean and neighboring countries, N Africa (?), Middle East, Central Asia, India	CL, VL, LR, PKDL, OL	***P. sergenti***
L. killicki Rioux, Lanotte et Pratlong, 1986	hyraxes	Kenya, Namibia, Tunisia	CL	***P. guggisbergi*** *P. saevus*
***L. major* complex**				
L. major Yakimoff et Schokhor, 1914	desert rodents, (*Hemiechinus auritus*, *Lepus tolai*, mustelids, dogs)	Middle East, Africa N of equator, Central Asia, India, Pakistan, NW China	CL, DCL, (VL)	***P. papatasi*** ***P. duboscqi*** ***P. salehi*** *P. alexandri* *P. ansari*
L. turanica Strelkova et Le Blancq, 1990	*Rhombomys opimus*	Middle Asia, Mongolia	–	(*P. papatasi*) (*P. andrejevi*) (*P. caucasicus*) (*P. mongolensis*)
***L. gerbilli* complex**				
L. gerbilli Wang, Qu et Guan, 1964	*Rhombomys opimus*	Middle Asia, S Mongolia	–	(*P. mongolensis*)
***L. aethiopica* complex**				
L. aethiopica Bray, Ashford et Bray, 1973	hyraxes, (*Cricetomys*)	Ethiopian highlands, Kenya, S Yemen (?)	CL, DCL, OL	***P. longipes*** ***P. pedifer***
***L. arabica* complex**				
L. arabica Peters et al., 1987	*Psammomys obesus*, (dogs)	Saudi Arabia	–	(*P. papatasi*)
New World species complexes				
***L. mexicana* complex**				
L. mexicana Biagi, 1953	*Ototylomys phyllotis*, (other forest rodents)	Yucatan, Belize, Guatemala, N Mexico, S Texas	CL, (DCL)	***Lu. olmeca olmeca*** ***Lu. yuphiletor***

***L. enrietii* complex**				
L. enrietii Muniz et Medina, 1948	*Cavia porcellus*	Parana State, Brazil	–	(*Lu. monticola*)
***L. amazonensis* complex**				
L. amazonensis Lainson et Shaw, 1972	*Proechimys* spp., (other forest rodents, marsupials, *Cerdocyon thous*)	The Amazon Basin, Brazil	CL, DCL, VL, PKDL, MCL	***Lu. flaviscutellata*, *Lu. olmeca nociva*** *Lu. ylephiletor*
L. aristidesi Lainson et Shaw, 1979	forest rodents, marsupials	Panama	–	(*Lu. olmeca bicolor*)
Venezuelan species**				
L. venezuelensis Bonfante-Garrido, 1980	?	Venezuela	CL	*Lu. olmeca bicolor*
L. garnhami Scorza et al., 1979	?, (*Didelphis marsupialis*)	Venezuelan Andes	CL	*Lu. townsendi* *Lu. youngi*
L. pifanoi Medina et Romero, 1959	?	Venezuela	DCL, (CL)	*Lu. flaviscutellata*
L. hertigi* complex**				
L. hertigi Herrer, 1971	*Coendou rothschildi*	Panama, Costa Rica	–	?
L. daenei Lainson et Shaw, 1977	*Coendou prehensilis*	Brazil	–	?
Newly described species				
L. forattini Yoshida et al., 1993	*Didelphis marsupialis*, *Proechimys iheringi*	Brazil	–	(*Lu. ayrozai*) (*Lu. yuilli*)
Subgenus *Viannia* Lainson et Shaw, 1987				
***L. braziliensis* complex**				
L. braziliensis Vianna, 1911	rodents, *Didelphis marsupialis*, *Choloepus didactylus*, (dogs, humans)	Brazil, Venezuela	CL, MCL	***Lu. wellcomei*** ***Lu. carerrai*,** ***Lu. intermedia*** ***Lu. whitmani*** ***Lu. trapidoi*** ***Lu. gomezi*** *Lu spinicrassa* *Lu migonei* *Lu pessoai* *Lu amazonensis* *Lu ayrozai* *Lu complexa* *Lu yucumensis* *Lu paraensis* *Lu llanosmartinsi* *Lu panamensis*
L. peruviana Velez, 1913	?, (dogs)	Peruvian Andes	CL	***Lu. verrucarum*** *Lu peruensis* *Lu aracuchensis*

***L. guyanensis* complex**				
L guyanensis Floch, 1954	*Choloepus didactylus*, *Tamandua tetradactyla*, (marsupials, rodents)	Guyana, Surinam, N Amazonian basin	CL	***Lu. umbratilis*** ***Lu. anduzei*** *Lu whitmani*
L panamensis Lainson et Shaw, 1972	Sloths, (*procyonids*, *monkeys*)	Panama, Costa Rica, Colombia	CL	***Lu. trapidoi*** ***Lu. gomezi*** ***Lu. yuilephiletor*** ***Lu. panamensis*** *Lu hartmanni* *Lu sangutnaria* *Lu ovallesi* *Lu shannoni*
L shawi Lainson et al , 1989	monkeys, sloths, procyonids	Amazonian Brazil	?	(*Lu whitmani*)
***L. naiffi* complex**				
L naiffi Lainson et Shaw, 1989	*Dasypus novemcinctus*	Amazonian Brazil	?	(*Lu paraensis*) (*Lu ayrozai*) (*Lu squamiventris*)
***L. lainsoni* complex**				
L lainsoni Silveira et al , 1987	*Agouti paca*	Brazil	CL	***Lu. ubiquitalis***
Newly described species				
L colombiensis Kreutzer et al , 1991	*Choloepus hoffmani*	Colombia, Panama	CL	***Lu. hartmani***
L equatorensis Grimaldi et al , 1992	*Choloepus hoffmanni*, *Sciurus granatensis*	Pacific coast of Equator	–	?

**Leishmania chagasi* is *L infantum* introduced into the New World in the Pliocene-Pleistocene era in infected canids or humans (Thomaz-Soccol et al 1993, Eisenberger & Jaffe 1999)

***Leishmania venezuelensis* not mentioned by Thomaz-Soccol et al 1993a, b, *L pifanoi* synonymized with *L mexicana* (Thomaz-Soccol et al 1993b), *L garnhami* synonymized with *L amazonensis* (Thomaz-Soccol et al , 1993b), all the species members of the *L mexicana* complex after Lainson & Shaw 1987

*** would be more appropriately classified as species of the genus *Endotrypanum* Mesnil et Brimont, 1908 (Croan & Ellis 1996)

[a] primary reservoir-host, (secondary or accidental host), ?, primary host unknown or unproven

[b] key to clinical manifestations of diseases CL, cutaneous leishmaniasis, DCL, diffuse cutaneous leishmaniasis, LR, leishmaniasis recidivans, MCL, mucocutaneous leishmaniasis, OL, oronasal or nasopharyngeal leishmaniasis, VL, visceral leishmaniasis, PKDL, post-kala-azar dermal leishmaniasis, AL, asymptomatic leishmaniasis, ( ), uncertain existence

CL chronic, painless, localized, single or multiple ulcerating "wet" or nodular nonulcerating "dry" lesions Self-limiting and self-curing, can leave disfiguring scars

DCL chronic, painless, fleshly, lepromatouslike nodules spreading locally and metastatically No self-cure, difficult to treat, tends to relapse

LR a sequel of CL, chronic, painless, discrete or coalescing, tuberculoidlike lesions spreading peripherally No self-cure, difficult to treat

MCL a sequel of CL, primary lesions, later metastatic spread to oronasal, pharyngeal, and anal mucosae with ulceration and progressive erosion of the soft tissue and cartilaginous structures No self-cure, difficult to treat, can relapse

OL reminiscent of MCL in the Old World

VL chronic fever, splenomegaly, hepatomegaly, lymphadenopathy, malaise, wasting, darkening of the skin, anemia, leucopenia, thrombocytopenia Death

can occur if untreated, spontaneous self-cure rarely
PKDL a sequel of VL appearing 6 months to one or more years after cure of VL Chronic dermal lesions without ulceration, either hypopigmented or erythematous macules at any part of body, which can become nodular Tendency to self-cure variable
AL asymptomatology based on parasitological evidence, the presence of parasites at some time either in the past or present
proven vector, parasites isolated and typed several times, man-vector and reservoir-vector contact established, experimental transmission in some cases,
strongly suspected vector, anthropophilic species, but only a few parasite isolations have been made and typed or parasites observed in the wild-caught sandfly and not typed
suspected vector, females morphologically indistinguishable from closely related species or parasites only observed in a blood-meal, experimental transmission in some cases,
( ), no transmission to man or transmission can occur, but the parasite is no-infective or non-pathogenic to man
P = *Phlebotomus*, Se = *Sergentomyia*, Lu = *Lutzomyia*
[d] The species *Leishmania chamaeleonis* Wenyon, 1921, *L. davidi* Strong, 1924, *L. henrici* Leger, 1918, *L. zmeevi* Andrushko et Markov, 1955, *L. sofieffi* Markov et al, 1964, *L. phrynocephali* Khodukin et Sofiev, 1940 and *L. helioscopi* Khodukin et Sofiev, 1940 were excluded from the subgenus *Sauroleishmania* as their life cycles (intestinal location in reptiles) do not correspond to the development of the *Leishmania* species from reptiles These species should be compared biochemically or genetically with those from the bloodstream and with accepted species of *Herpetomonas* Kent, 1880 and *Leptomonas* Kent, 1880 before considering them to be congeneric with *Leishmania* species (Telford 1995)

nia. The most important disease vectors are listed in Tab. 1. The other three recognized genera are *Chinius* Leng in the Old World and *Warileya* Hertig and *Brumptomyia* França et Parrot in the New World (Lane 1993).

Females lay eggs in moist soil, among leaf litter, in the animal burrows and termite hills, in the bark of old trees, soil or wall cracks, ruined buildings and household rubbish or stable floors. In such habitats, larvae find food (various kinds of organic matter), heat and humidity necessary for development. Similarly to other Nematocera, larvae are eucephalic and apodal. There are four larval instars. In temperate or arid areas, the fourth instar larvae can undergo diapausis. Adult sandflies of both sexes feed on plant sugars, females, in addition, need a blood meal for ovarian development. They suck blood from a variety of vertebrates, mainly from mammals but also from reptiles and amphibians, a few species feed on birds. Biting is usually restricted to crepuscular and nocturnal periods, although in forests or darkened rooms females bite throughout the day. Sandflies are weak fliers, they fly in a series of characteristic hops. Their flight range is limited to a few hundred meters from the breeding site.

*P papatasi** and some other species can be a nuisance pests owing to irritation from their bites. Besides of *Leishmania* parasites, sandflies are vectors of a kinetoplastid protozoan *Endotrypanum*, a parasite of sloths. They transmit the bacterium *Bartonella baciliformis*, agent of the Oroya fever (also called Carrion's disease) in Peru, Ecuador and Colombia (transmitted by *Lu verrucarum**) and several viruses (phleboviruses, vesiculoviruses). The most important are phleboviruses causing sandfly fevers (papataci fever), common in the Mediterranean region and the Middle East and transmitted principally by *P. papatasi* (Lewis 1973, Lane 1993, Service 1996).

PART IV.
Geographical distribution: vector-parasite-host association

The distribution of *Leishmania* spp. is a result of long-termed complex interaction between the parasite, the vector and the host. Therefore, the ecology and epidemiology of the leishmaniases are extremely diverse. The most detailed reviews of the epidemiology of New World and Old World leishmaniases were published by Shaw & Lainson (1987) and Ashford & Bettini (1987), respectively.

Leishmania spp. are essentially parasites of wild animals. In the Old World, various species of rodents, carnivores and hyraxes are involved. In the New World, the spectrum of reservoirs is enlarged with marsupials, edentates, procyonids and monkeys (Tab. 1). Most natural hosts tolerate infections, that often remain benign and inapparent. This is advantageous for *Leishmania* spp. in countries in which the climate is not suitable for sandflies during all year and, therefore, the overwintering of the parasite must occur in the reservoir hosts. Survival of *Leishmania* in their hosts for such a long period suggests minimal antileishmanial activity against the parasite by these hosts (Schnur & Greenblat 1995).

The habitat preference of vectors and hosts and a degree of overlap of their habitats leads to a range of various parasite strategies seen in nature. The Fig. 4 is a diagrammatic summary adopted from Shaw & Lainson (1987) which shows how the relationships of habitats determine the possibility of disease transmission. The first three situations (a–c) represent enzootic cycles and the following three diagrams are zoonotic cycles in which man can be an accidental host (R_2). The situation (d) possibly applies to *L. amazonensis* and the situation (e) to *L. guyanensis* and *L. panamensis*: man becomes infected at ground level, while the enzootics among sloths occur in the canopy. The

* P = *Phlebotomus*, L = *Leishmania*, Lu = *Lutzomyia*

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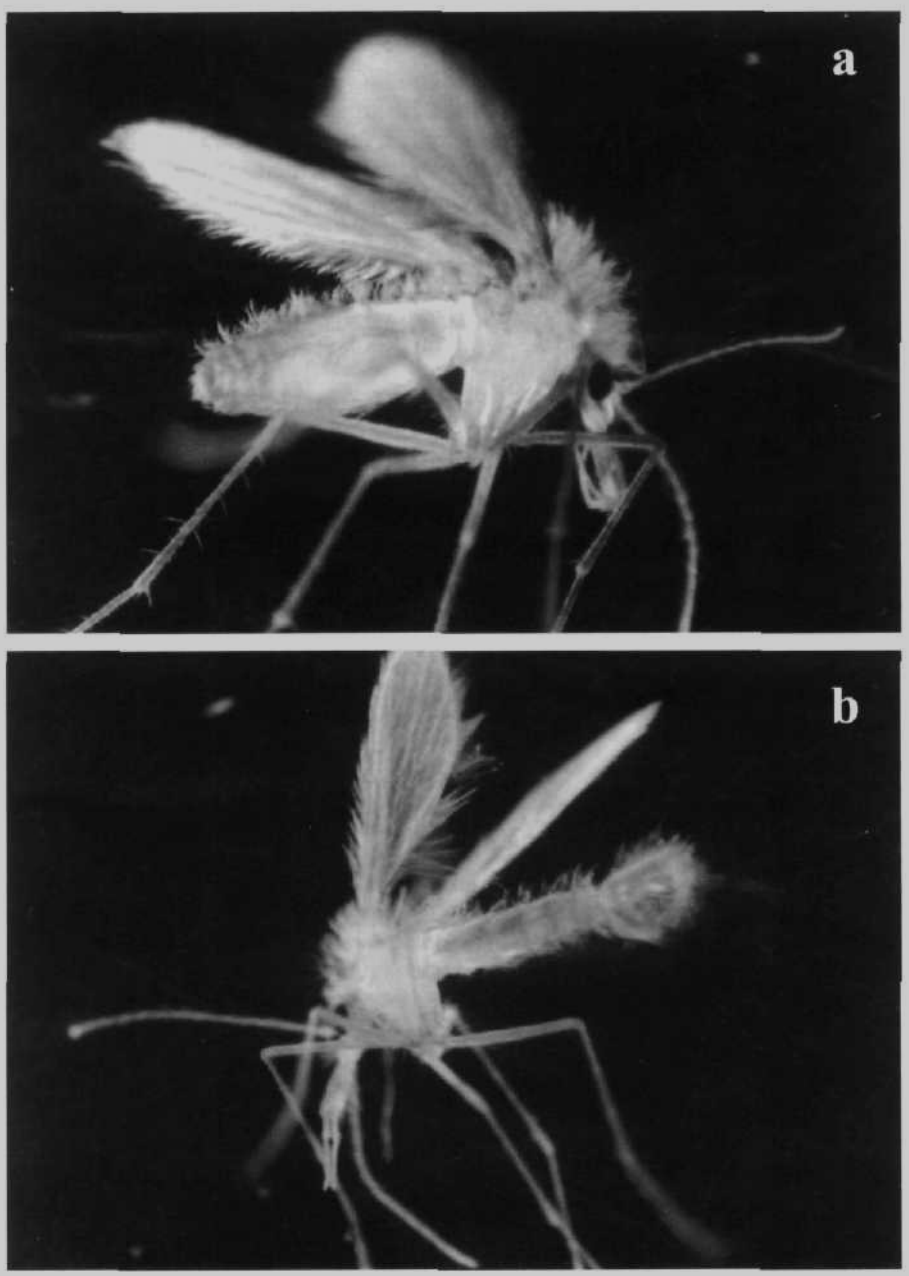

Fig. 3. Female (a) and male (b) of the sandfly *P. papatasi*, a vector of *L. major*. Typical appearance of abdomen distinguishes the sexes. Male terminal abdominal segments with external genitalia are important taxonomical characters.

last possibility is supposed to occur in *L. guyanensis* as anteaters are probably infected by the bite of a secondary vector.

Shaw (1997) believes that the major factor controlling the distribution of *Leishmania* spp. in mammals is probably the biting habit of sandflies. He argued that vectors exert greater selective pressure on the parasite than mammalian hosts: only a few *Leishmania* species, like *L. enriettii* restricted to guinea pigs, developed a high degree of host specificity. On the other hand, there are hosts (e. g. man), which are susceptible to infections by many species of *Leishmania* whose natural hosts range from edentates to rodents. Similarly, the golden hamster is not a natural reservoir of any *Leishmania* species but is susceptible to most of the species that infect man. In some cases, however, the distribution of a suitable host rather than that of a vector governs the parasite (Schnur & Greenblat 1995). In Israel, *P papatasi* is widely distributed, but CL is contracted only by people visiting very specific areas, those in which *Psammomys obesus* and *Meriones crassus* occur (Schle-in et al. 1984)

The example of *L. major* was used by Schnur & Greenblat (1995) to show that both host and vector specificity does not occur at the species level. This parasite is distributed over a wide geographical range with at least 15 reservoir rodent species and 3 vector species in different parts of the range. However, a detailed study on Central Asian foci of *L. major* (reviewed by Strelkova 1996) showed that the two reservoir host species present in this foci highly differ in the susceptibility to the infection. In *Rhombomys opimus* ulceration and visceralization never developed and infections lasted on average for 7 months with self healing in nearly 90% cases. However, in *Meriones libycus* the infection led to a rapid ulceration and lasted, on average, for five months. Also, the infection rate of *M. libycus* in nature is low (3–4%). Similar situation can be expected in other parts of the *L. major* range: despite the parasite being isolated from a broader spectrum of species, the number of true reservoirs can be far lower.

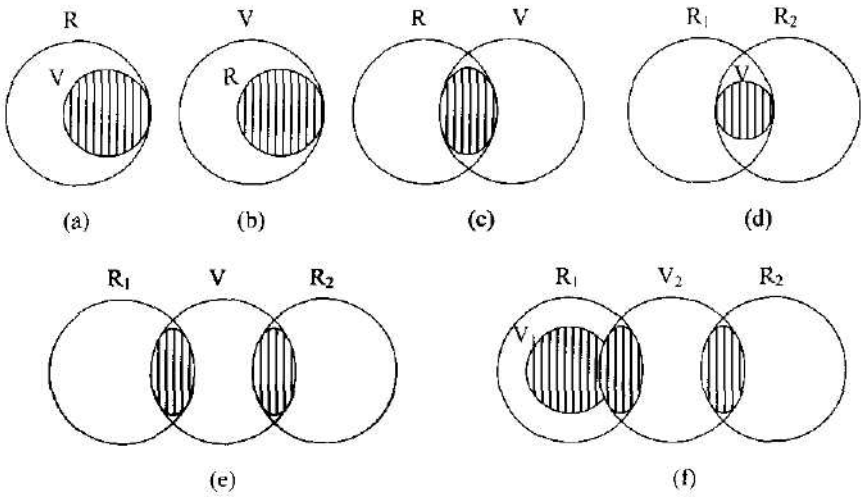

Fig. 4 The relation of vector (V) and reservoir (R) habitats to transmission (shared areas) Adopted from Shaw & Lainson (1987) (a) transmission throughout vector habitat, (b) transmission throughout reservoir habitat, (c) transmission limited to a small area of habitat overlap, (d) transmission to two hosts with a little different habitat preferences throughout the habitat of the vector, (e) transmission outside the habitat of primary host by the same vector, (f) transmission by a secondary vector to a secondary host outside the habitat of the primary hosts and vector R_1, primary reservoir, R_2, secondary reservoir. V_1, primary vector, V_2, secondary vector

Table 2. Suggested functions of gp63 and LPG in the host

	Gp63	LPG
Inhibition of chemotaxis of monocytes and neutrophils	(Sorensen et al 1994)	(Lo et al 1998, Frankenburg et al. 1990, 1992)
Direct binding to CR3 receptor, mannose-fucose receptor and fibrinogen receptor	(Russell & Wright 1988, Wilson & Pearson 1986, Rizvi et al 1988)	(Talamas-Rohana et al 1990, Kelleher et al 1995, Wilson & Pearson 1986)
A C3 acceptor in the uptake of *Leishmania* by macrophages via the CR3 receptor	(Russell 1987)	(Da Silva et al. 1989)
Conversion of C3b to iC3b and, therefore, exploitation of opsonic properties of complement while avoiding its lytic effects	(Brittingham et al. 1995)	
A barrier preventing the insertion of MAC complexes		(Puentas et al. 1990)
Protection from intracellular degradation in the phagolysosome	(Chaudhuri et al 1989, Scay et al. 1996, McGwire & Chang 1994, Sorensen et al 1994)	(McNeely et al 1989, McNeely & Turco 1990, Proudfoot et al. 1995, 1996)
Lesion formation in the host via a degradation of the extracellular matrix	(McMaster et al. 1994)	
Cleavage of CD4 on T cells	(Hey et al. 1994)	
Suppression of cytokine expression in monocytes		(Frankenburg et al. 1990, Hatzigeorgiou et al 1996)
Inhibition of phagosome-lysosome fusion		(Desjardin & Descoteaux 1997)

Also in other aspects, these Central Asian foci are a good example of a complicated epidemiological situation. *Rhombomys opimus* in this area is infected by three *Leishmania* spp.: *L. major*, *L turanica* and *L. gerbilli*; the only species pathogenic for humans being *L major*. *L turanica* is widespread in *Rhombomys opimus* throughout the region. In southern part of *Rhombomys opimus* range, *L turanica* existed sympatrically with *L. major* and *L. gerbilli*. The rate of *Rhombomys opimus* infection by *L turanica* is stably very high (50–100%). The infection rate of *Rhombomys opimus* by *L major* is very low in the beginning of the transmission season, but in early autumn it attains 50% or more. *L. gerbilli* is ever rarer in *Rhombomys opimus* as compared with *L major;* this species is restricted to the region of the Emba river. High proportions of *Rhombomys opimus* are infected with more than one *Leishmania* species. In fact, no pure *L. major* isolates could be obtained from the host, but only *L. major–L. turanica* mixtures and, in two cases, the presence of all three parasite species in the same individual rodent was described (Strelkova 1996).

Humans and dogs are found infected in many regions but they are only secondary hosts of most leishmania (Lainson & Shaw 1987). The only *Leishmania* spp. for which humans are considered to be primary reservoir hosts are *L. donovani* in India and, less certainly, *L tropica* in different regions. Dogs are not considered to be reservoirs of *L. tropica* and the presence of *L. major* in dogs is only incidental. They are important domestic (secondary) hosts of *L. infantum* and *L. chagasi*, and, maybe, also of *L. braziliensis* and *L. peruviana*. Primary hosts in rural foci of *L. infantum* and

L. chagasi are wild canids: the jackal *Canis aureus*, the wolf *Canis lupus* and the fox *Vulpes vulpes* in the Old World and *Lycalopex vetulus* and *Cerdocyon thous* in the New World (Ashford & Bettini 1987, Schnur & Greenblat 1995). It should be noted that *L. chagasi* was introduced into the New World in infected humans or dogs during historic times (Schnur & Greenblat 1995, Eisenberger & Jaffe 1999) and, therefore, the adaptation to New World canids must be a recent event.

PART V.
Leishmania life-cycle

A. Development in the sandfly vector

1. Mode of development – implications for Leishmania *taxonomy*

Leishmania spp. undergo development in the gut of the sandfly. This development (a) is restricted to the hindgut causing contaminative transmission, or (b) the hindgut development is followed by migration of promastigotes to the midgut and foregut causing transmission by bite or (c) the development takes place only in the midgut and foregut with transmission by bite. Lainson & Shaw (1979) placed emphasis on the mode of development and divided phlebotomine species into three sections (Fig. 5): Hypopylaria, Peripylaria and Suprapylaria, respectively, these sections having no taxonomic status. Lainson & Shaw (1987) later erected the subgenus *Viannia* for the species of the Section Peripylaria, retaining the subgenus *Leishmania* Saf'janova, 1982 for the species of the Section Suprapylaria. The reptilian parasites with both hypopylarian and peripylarian development were included in the genus *Sauroleishmania*.

The same authors argued that the mode of development enables a natural grouping of the leishmanial parasites and perhaps, therefore, reflects their phylogenetic relationships (Lainson et al. 1977, Lainson & Shaw 1987). The hindgut development was considered to be the ancient mode while the parasites from the section Suprapylaria were interpreted like the most derived ones, which have lost the primitive hindgut development. This hypothesis is logically consistent with the assumption that *Leishmania* parasites evolved from monogenetic parasites of invertebrates, which also undergo hindgut development with passing of resistant, infective forms with excreta. Also the primitive nature of the peripylarian parasites (subgenus *Viannia*) in comparison with the suprapylarian ones (subgenus *Leishmania*) is in accordance with the assumed evolution of the genus (see the Part II). Peripylarian parasites are restricted to the Neotropics, a suggested area of the origin of the genus, and they are often found in edentates, the ancient group of eutherian mammals which evolved at the turn of Mesosoic/Cenosoic period with the highest species richness in Pliocene and Pleistocene, representing possibly the earliest hosts of *Leishmania*.

In view of current information, however, the evolutionary significance of the development in sandfly should be interpreted with caution. Firstly, the mode of development can be influenced by a number of extrinsic factors and is more flexible, than was suggested (Schlein 1986, Añez 1989, Walters 1993). Secondary, the application of modern taxonomic methods leads to the revision of the phylogenetic relationships between the members of the genus *Leishmania*. Molecular analyses showed, for example, that *L. hertigi* with the suprapylarian development is the most divergent of all *Leishmania* species studied by Croan & Ellis (1996), and is close to the *Endotrypanum* lineage. However, this argument can not be as strong in view of the fact, that natural vector of *L. hertigi* is still unknown and experimental infections were done with *Lu. longipalpis* and *Lu. truncata* (Lainson & Shaw 1987). Additionally, a significant DNA-based evidence (reviewed by Noyes et al. 1998) established the subgenus *Sauroleishmania* with hindgut development to be a sister group of the subgenus *Leishmania*, separated after the divergence of the subgenus *Viannia*. Therefore, it seems that several transitions between the modes of development have occurred during the phylogeny of

the genus. The simplest possible scenario is shown in Fig. 6, but additional studies on natural combinations of vectors and parasites and detailed phylogenetic trees are necessary to obtain plausible information about the evolutionary significance of this trait.

The following text concerns only on the parasites with suprapylarian and peripylarian patterns of development which are infective for mammals.

2. Midgut infections

The complex development of *Leishmania* spp. in the vector was described only in some natural parasite-vector combinations, mainly because to find naturally infected flies is rare and the establishing and raising of sandfly colonies is difficult. Most valuable studies were done on natural systems: *L. panamensis* in *Lu. gomesi* (Walters et al. 1989a), *L. chagasi* in *Lu. longipalpis* (Walters et al. 1989b, Elnaiem et al. 1992) and *L. major* in *P. papatasi* (Warburg et al. 1986, Killick-Kendrick et al. 1988). A general pattern of the development was established as follows (reviewed by Killick-Kendrick 1979, Walters 1993, Schlein 1993, Schnur & Greenblat 1995).

A bloodmeal containing **amastigotes** (Fig. 7) is quickly surrounded by the peritrophic matrix, a chitinous framework with a protein-carbohydrate matrix, which is secreted by epithelial cells of the abdominal midgut. In ingested bloodmeal, amastigotes usually divide and then differentiate into

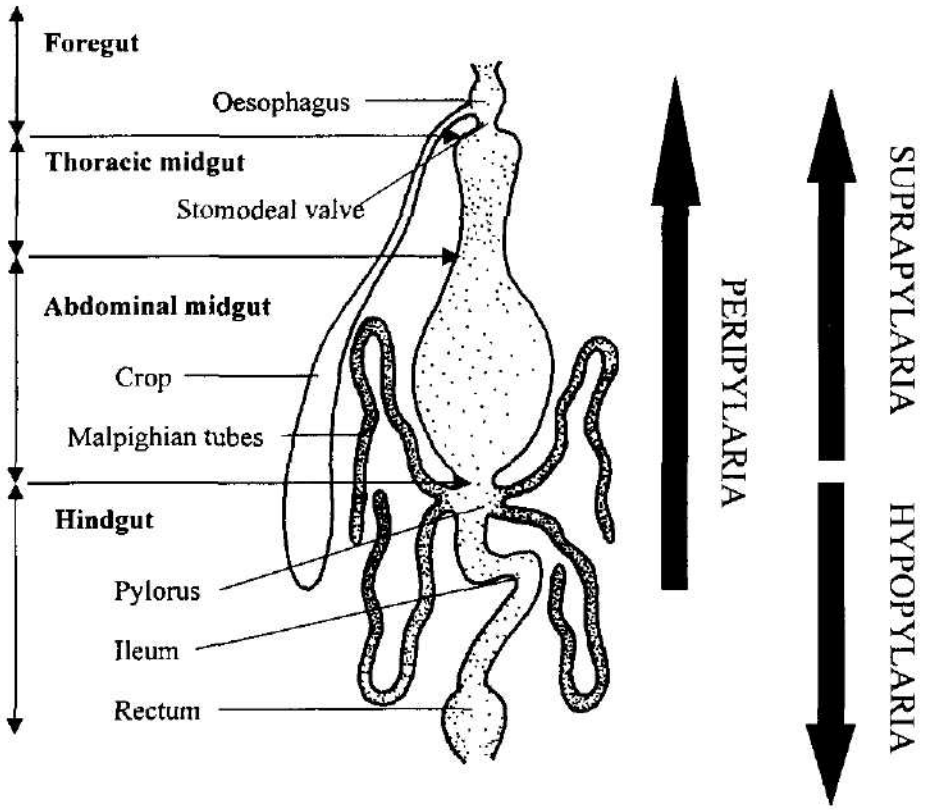

Fig. 5 Gut morphology of sandflies with illustration of three recognised modes of development in the genus *Leishmania* (according to Lainson & Shaw 1987)

flagellated promastigotes, called by Walters (1993) **stumpy promastigotes**, which then transform or enlarge into long, slender **nectomonad promastigotes** After the breakdown of the peritrophic matrix (about 3 days post the meal), nectomonads escape into the ectoperitrophic space of the midgut lumen Schlein et al (1991) found in *L major- P papatasi* system, that the disintegration of the anterior end of the peritrophic matrix is catalyzed by chitinolytic enzymes of *Leishmania* Once inside the midgut, promastigotes attach by the tip of flagellum among the microvilli of epithelial cells The attachment is reversible and it prevents the promastigotes from being expelled with undigested bloodmeal remnants The peripylarian parasites do not attach in the abdominal midgut but migrate straight out to the hindgut In suprapylarian spp , promastigotes repeatedly attach and detach, and rapidly multiply in the abdominal midgut, and afterwards move anteriorly Walters (1993) described that the elongated nectomonads transform into **short nectomonad promastigotes**, which are predominant forms in the thoracic part of the midgut, called also cardia, 5–7 days post bloodmeal Short promastigotes were proposed by the author to be precursors to **metacyclic promastigotes**, small, highly motile forms with a long flagellum, which are infective for the mammalian host In culture, these forms are produced in the stationary phase of growth

The described morphogenesis is coincident with developmentally regulated changes in expression of main surface glycoconjugates of the parasite, the **lipophosphoglycan (LPG)** and the **surface protease gp63** (Davies et al 1990) There are two hypotheses concerning the role of leishmanial LPG in the vector The excreted form of LPG (EF, excreted factor) was described to be involved in **a protection from hydrolytic damage by the gut proteases** by modulating their activity during bloodmeal digestion (Schlein et al 1990) However, results of Saraiva et al (1995) argued against this role of LPG they reported a delay in LPG expression on the surface of *L major* in *P papatasi* until the appearance of nectomonads on day 3, while the peak of protease activity in this sandfly species occur at 24–34h post bloodmeal (Dillon & Lane 1993a) The expression, however, coincides with the time of escape of promastigotes from the peritrophic matrix, which supports the second proposed role of LPG in the sandfly, **the attachment to the midgut wall** (Pimenta et al 1992, Sacks et al 1994) Dillon & Lane (1999) described a 65 kDa microvillar protein which probably acts as a ligand for the parasite's LPG The attachment is developmentally regulated by a modification of LPG which accompanies the transformation of promastigotes from procyclic to metacyclic forms Particularly, it is the substitution of terminally exposed galactose residues by arabinose in *L major* (Pimenta et al 1992) and folding and clustering of the extended phosphoglycan chains in *L donovani* (Sacks et al 1995) As a result, the infective metacyclic promastigotes lost the property to bind to the midgut epithelium and are selectively released for subsequent transmission by bite

The role of gp63 in the sandfly vector is probably the acquisition of nutrients from the bloodmeal Proteolytical degradation of haemoglobin could provide both amino acids (especially prolin) used as the primary energy source of promastigotes (Mukkada 1985) and haem, which they cannot synthesize (Chang & Chang 1985) The nutritional role of gp63 in the vector is indirectly supported by the fact that surface metalloproteases similar to leishmanial gp63 were found in monoxenous insect trypanosomatids *Crithidia* and *Herpetomonas* (Etges 1992) The digestion of haemoglobin in the vicinity of the parasites inside the peritrophic matrix may have an additional effect the presence of haemoglobin inhibits a secretion of parasite chitinases (Schlein & Jacobson 1994a) Therefore, its break-down is a prerequisite for the function of these enzymes and, consequently, for lysis of the peritrophic matrix (Schlein 1993)

3 Foregut and/or hindgut infection

Suprapylarian parasites transform in the vicinity of a stomodeal valve (Fig 7) from free-swimming nectomonads to non-motile shorter and broader **haptomonad stages** Some haptomonads colonize the oesophagus and pharynx, where are found also attached **paramastigotes** (the forms distin-

guished from promastigotes by a juxtanuclear kinetoplast, the free-swimming ‚nectomonad' phase of paramastigotes could be seen already in the midgut) Both haptomonads and paramastigotes attach to the cuticle lining of stomodeal valve and foregut by the flagellum which forms broad, hemidesmosome-like plagues Lumenal free-swimming slender promastigotes are most likely metacyclic forms From the pharynx, the infection may spread forwards to the cibarium and in some systems, metacyclic promastigotes are found even in the proboscis (Killick-Kendrick 1979, Schlein 1993, Walters 1993)

The initial establishment of infection in peripylarian spp is in the pylorus and the ileum of the fly In these parts of the hindgut, haptomonads and paramastigotes attach to the cuticular surface by hemidesmosomes and multiply Motile promastigotes which migrate to the thoracic midgut are produced. Some parasites migrate straight to the thoracic midgut without going through the hindgut phase Further development is similar like in the suprapylarian spp (Killick-Kendrick 1979, Walters 1993)

4 Transmission

The location of parasites in the sandfly digestive tract is of crucial importance for the transmission to the vertebrate host Transmission by bite takes place in suprapylarian and peripylarian species, which colonize the foregut. In this respect, two main hypotheses have been suggested either the parasites emerge from an infected sandfly during feeding only if the proboscis itself is infected (Adler & Theodor 1935) or they are regurgitated with a backflow of ingested blood Originally, the regurgitation was supposed to result from a mechanical block of the foregut (Shortt & Swaminath 1928) or stomodeal valve (Warburg & Schlein 1986) by the parasites More recently, the damage to the chitin layer of the stomodeal valve by leishmanial chitinolytic enzymes was proposed to be the cause of regurgitation of parasites from the thoracic midgut (Schlein et al 1991, 1992) This hypothesis also offers the explanation of multiple probing by infected flies and the difficulty they

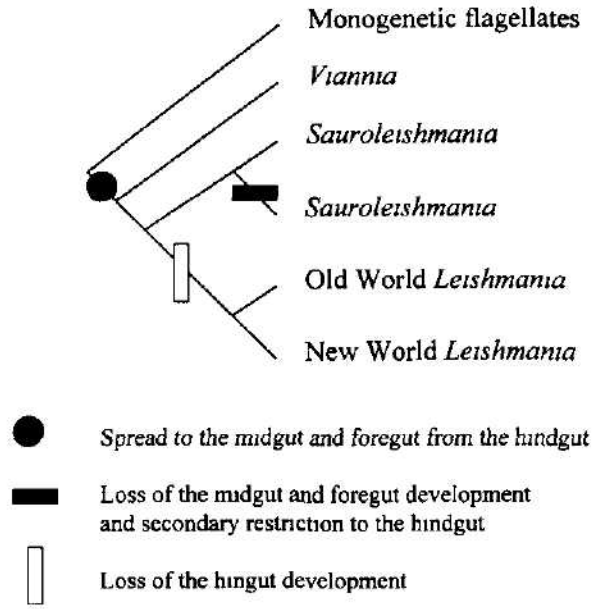

Fig 6 Suggested evolutional events in modes of development of the genus *Leishmania* in sandfly vectors

have in obtaining a full blood meal (Killick-Kendrick et al 1977) A third possible way of transmission, the inoculation of parasites into the host skin with sandfly saliva, is supported by the study of Killick-Kendrick et al (1996), who found metacyclic promastigotes of *L tropica* invading salivary glands of *P duboscqi* Therefore, there is perhaps more than one mechanism of transmission by bite (Killick-Kendrick et al 1996)

Contaminative transmission by ingestion of infected sandfly takes place in the genus *Sauroleishmania* (Lainson & Shaw 1987) In mammals, the contaminative route of transmission is probably an occasional, less frequent alternative It probably occurs in transmission of *Leishmania* to dogs which regularly lick off the flies engorging on their nose and breathe with the mouth open In man, contaminative transmission may occur when a heavily infected biting fly is crushed on the skin (reviewed by Killick-Kendrick 1979)

5 Factors affecting susceptibility of sandflies to Leishmania

The *Leishmania*-sandfly interaction is affected by many factors, which have either adverse or beneficial effects on the course of infection (for review see Killick-Kendrick 1979) Intrinsic factors, such as proteolytic enzymes (Dillon & Lane 1993a) and sandfly lectins (Wallbanks et al 1986, Volf et al 1994) make each sandfly species a unique habitat for leishmania parasites This habitat is further influenced by many exogenous factors, particularly by temperature (Leaney 1977) and the two types of food, blood and sugar solutions, taken by sandfly females

The main sources of sugars in nature are nectar, fruit and plant saps and, above all, honeydew This is a sugary solution containing also amino acids, excreted from the anus of aphids, coccids and other plant-sucking insects The beneficial effect of sugars on the parasite development is evidenced by the fact that experimental transmissions were not successful until sugars were offered to infected females (reviewed by Killick-Kendrick 1979) As shown later (Schlein et al 1993), the sugarmeal modifies the functioning of parasite chitinases which damage the stomodeal valve and, therefore, facilitate the *Leishmania* transmission However, sandflies feed selectively on different sugar sources (Schlein and Warburg 1986), some of which, on the contrary, may impair leishmanial infections In the experiments of Schlein & Jacobson (1994b), feeding on *Malva nicaeensis* and on the honeydew excreted by *Icerya purchasi* produced vital and thriving infections, whereas diets of *Ricinus communis*, *Capparis spinosa* or *Solanum luteum* reduced the numbers of parasites

Sugar diet can alter the gut environment also indirectly, by bacterial or fungal contamination, which negatively influence not only the parasite, but also the fly (Killick-Kendrick 1979, Schlein et al 1985) Therefore, it was suggested that defence mechanisms evolved in sandflies to decrease the probability of intensive gut contamination Sugar meals taken from leaves and stems by piercing (‚sterile' meal) directly enter the midgut like the blood (also ‚sterile'), while meals taken from the plant surface (possibly contaminated) are located in the crop The crop contains an antibacterial agent which influences the meal before its gradual release to the midgut (Schlein & Warburg 1985, Schlein & Warburg 1986) This hypothesis was not supported by the study of Añez et al (1997) who used capillary feeding (mimicking the piercing) and found that the destination of a meal depends on the salt concentration of the fluid rather than the feeding mode of the fly However, the number of flies in their experiments were not sufficient for a definite conclusion

The development of leishmania in the sandfly is also considerably affected by the blood source Schlein et al (1983) described inhibition of experimental leishmanial infection in *P papatasi* fed on turkey blood Flies inhabiting burrows of *Psammomys obesus* near turkey sheds were uninfected in a strong contrast with females from burrows in other sites (Schlein & Jacobson 1994c) The same lethal effect was reported by the same authors for chicken blood In this respect, there is also a strong difference among different mammalian species the percentage of heavy infections of *L major* in *P papatasi* is considerably decreased in flies fed on human blood in comparison with

those fed on the blood of *Psammomys obesus* (Schlein & Jacobson 1996) Blood from different mammals may cause different effects on the secretion of sandfly midgut proteases (Schlein & Jacobson 1998) In addition, the bloodmeal might affect the parasite indirectly through the lectin-like activity in the sandfly gut These lectin-like molecules bind to LPG (Palanova & Volf 1997) and agglutinate *Leishmania* promastigotes (Wallbanks et al 1986, Svobodova et al 1996) Secretion of the midgut agglutinin is increased after bloodfeeding (Volf & Killick-Kendrick 1996, Volf & Palanova 1996) and specific inhibition of lectin activity increases leishmanial infection (Volf et al 1998)

As shown above, the habitat inside the sandfly gut undergoes temporal changes during the life of the individual fly, and it differs also between populations of one sandfly species with variable feeding preferences and food sources in various geographical areas Despite of this variability, there is more or less close fit between *Leishmania* spp and their vectors, which is presumably the result of their coevolution Proven vector of one parasite species is often unable to support the full development of another species The taxonomic relationships of Old World vectors suggest that the coevolution results in a restriction of the range of sandflies in which the parasite species can readily develop For example, *L major* is transmitted by species of the subgenus *Phlebotomus* Rondani, *L tropica* by *Paraphlebotomus* Theodor, *L aethiopica* and *L infantum* by *Larroussius* Nitzulescu, *L archibaldi* by *Synphlebotomus* Theodor and *L donovani* by *Paraphlebtomus* and *Euphlebotomus* Theodor (Killick-Kendrick 1985, Killick-Kendrick 1990)

The characters which determine the susceptibility or refractoriness of sandflies to leishmanial infection are controlled genetically, but multiple genes are involved A completely refractory lines of *P papatasi* to infection with *L major* were not obtained despite 17 consecutive generations of selection (Wu & Tesh 1990a, b) Similar results were obtained by Killick-Kendrick on *P duboscqi* infected with *L tropica* (pers comm) There are now two hypotheses concerning the mechanism of **species specificity in the parasite-vector relationship**, both involving the surface LPG (see also Part VA2)

Schlein (1986) suggested that the mechanism favouring the successful growth of *Leishmania* in the gut of sandflies is the suppression of digestive enzymes by the parasite in its natural vector The level of proteolytic enzymes was reduced in *P papatasi* infected with promastigotes of the natural parasite, *L major*, and elevated by the unnatural parasite, *L donovani* (Borovsky & Schlein 1987) Similar results were obtained with amastigote-initiated infections (Dillon & Lane 1993b) Further search for the mechanism of protection led to the discovery of the role of species-specific surface LPG in its excreted form (EF) The addition of EF of *L major* significantly increased survival of LPG-defective strain of *L major*, while addition of EF of *L donovani* did not promote the survival (Schlein et al 1990)

The second hypothesis is based on the role of LPG as a ligand to sandfly midgut epithelium Due to the extensive interspecific polymorphism of LPG, the attachment might contribute to species-specific differences in vectorial competence (Pimenta et al 1994, Sacks et al 1994) It was shown that only *L major* strains having the complete procyclic LPG could attach to the midgut of their natural vector, *P papatasi* The second sandfly tested, *P argentipes*, was permissive not only to *L donovani* (natural vector) but also to *L major*, *L tropica* and *L amazonensis* (unnatural combinations) Similar preliminary results are reported also for *Lu longipalpis* The authors suggest that *P argentipes* (and *Lu longipalpis*) midguts possess a receptor, lacking in *P papatasi*, for a relatively conserved oligosaccharide on procyclic LPGs The natural selection in *L major* led to the expression of an unusual, galactose-substituted LPG able to bind to the midgut of *P papatasi* through an other, unique receptor It provides them with the advantage of a ‚free niche' a colonization of the widely distributed sandfly species, *P papatasi*, which is refractory to other parasites (Pimenta et al 1994, Sacks et al 1994)

In this respect, it would be interesting to characterize the LPG of other *Leishmania* species known to be transmitted by *P papatasi* (*L turanica*, *L arabica*, *L tarentolae*) and assess the vector competences of the other two natural vectors of *L major*, *P duboscqi* and *P salehi* (Jacobson 1995) The susceptibility of *P duboscqi* to *L tropica* was already shown by Killick-Kendrick et al (1994) Schlein & Jacobson (1998) disagree with the interpretation of Pimenta et al (1994) In their experiments (Schlein & Jacobson 1998) with *L donovani* and *P papatasi*, the crucial period for the success of the infections was during blood digestion Afterwards, the attachment of *L donovani* to the epithelium was indistinguishable from that of *L major*

Regardless of the exact mechanism, the vectorial competence is often tested in laboratories In this respect, it should be mentioned that experimental infections with unnatural vector-parasite combinations are often more successful, than expected (Killick-Kendrick et al 1977, Lawyer et al 1987, Ryan et al 1987, Walters et al 1987, Rangel et al 1993, etc) It may be an artifact of the laboratory conditions including an abnormally large numbers of parasites offered to sandflies Therefore, the assessment of vector competence should always combine experimental and field data like geographical distribution and association with endemic regions, habitat preference, biting behavior and host preferences (anthropophily vs zoophily)

B. Development in the vertebrate host

1 The parasite-macrophage interaction

The most intriguing aspect of *Leishmania*-host association is the ability of amastigotes to live in mononuclear phagocytes, mammalian cells specialized in defence against invaders Although macrophages and monocytes are well equipped to internalize, kill and digest invading pathogens, *Leishmania* spp are able to counteract their degradation activities During the adaptation to the intracellular life, these parasites developed multiple strategies how to evade host's defence system Four sequential events are essential for the establishment of the intracellular life recognition, entry, survival and multiplication (Chang 1983)

1 1 Recognition and entry

After deposition in the skin by the bite of sandflies, promastigotes face two effective immune mechanisms of the mammalian host lysis by complement and destruction by phagocytes In a blood pool, induced by the sandfly bite, promastigotes immediately encounter host serum and activate complement via the classical pathway, alternative pathway or lectin pathway (reviewed by Mosser & Brittingham 1997) **Complement activation** leads to the formation of the complement cleavage product C5a which attracts macrophages by chemotaxis Monocytes from the blood move in the direction of the chemotaxin gradient to the area of infection and start to interact with promastigotes (Bray 1983) The other effect of complement activation is the opsonisation of the parasite by complement protein C3b Surface bound C3b can act as a ligand for macrophage complement receptors (CR) which results in enhanced uptake of the parasite into phagocytic cells On the other hand, C3b can also initiate the formation of membrane attack complex (MAC) mediating parasite cell lysis (Pearson & Steigbigel 1980, Mosser & Edelson 1984)

Leishmania promastigotes developed a unique defensive mechanism how to avoid the destruction by complement, while exploiting its opsonic properties Metacyclic promastigotes resist the complement-mediated lysis due to the function of two main surface molecules, promastigote surface protease gp63 and lipophosphoglycan LPG It was shown that C3b is converted by gp63 to inactive form iC3b, which remains opsonic, but is unable to support the formation of MAC (Brittingham et al 1995, Brittingham & Mosser 1996) The role of LPG in serum resistance is most likely mechanical, elongated LPG in metacyclic promastigotes may act as a barrier preventing the inser-

tion of MAC complexes (Puentas et al. 1990). In addition, leishmanial protein kinases have been reported to inactivate complement components by phosphorylation (Hermoso et al. 1991).

Phagocytosis of *Leishmania* by macrophages is a receptor-mediated event which involves multiple macrophage receptors, parasite surface proteins and host serum factors (reviewed by Mosser & Brittingham 1997). **Serum dependent adhesion** consists in binding of C3b opsonised promastigotes to macrophage complement receptors. Da Silva et al. (1989) identified in *L. major* the CR1 receptor for C3b as the principal molecule involved in the binding of serum opsonised metacyclic promastigotes. In their experiments, CR1-mediated uptake of metacyclic parasites did not generate respiratory burst in the macrophage whereas CR3-mediated uptake of promastigotes from log-phase cultures triggered a respiratory burst. More recently it was demonstrated that CR1 and CR3 cooperate in a unique manner in binding both procyclic and metacyclic promastigotes of *L. major* and that not CR1 but CR3 is the primary receptor involved in the complement-mediated phagocytosis of *Leishmania* (Sutterwala et al. 1996, Rosenthal et al. 1996). The benefit of utilizing both CR1 and CR3 receptors is that they do not elicit respiratory bursts.

Besides the opsonin facilitated, i. e. serum dependent binding, there is also a serum independent **direct binding** of promastigotes to macrophages that contributes to parasite attachment and internalization. Direct binding involves interactions of parasite carbohydrates and macrophage lectin-like receptors, mainly mannose/fucose receptor, receptor for advanced glycosylation end products (AGE) and β-glucan receptor (Blackwell 1985, Wilson & Pearson 1986, Mosser et al. 1987, Mosser &

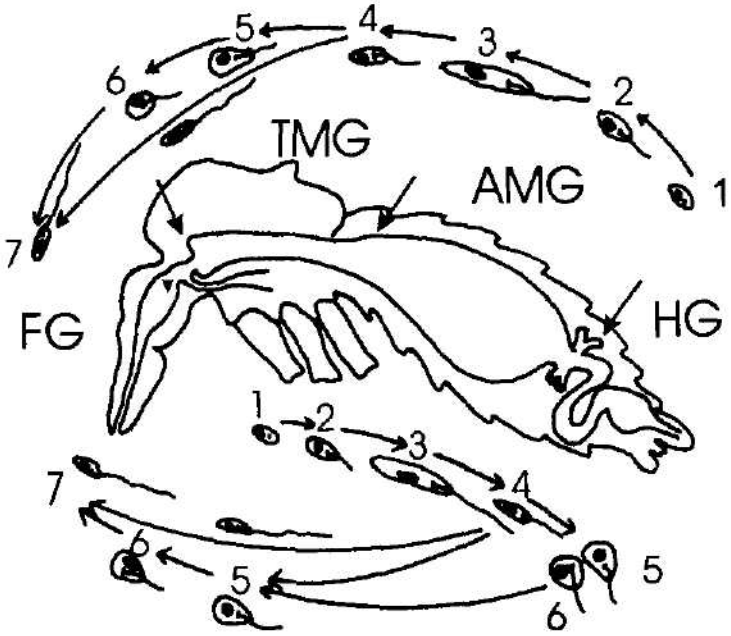

Fig 7 Generalized scheme of the morphological development and localization of suprapylarian (above) and peripylarian (below) *Leishmania* spp in sandflies. Arrows divide the gut to: FG, foregut; TMG, thoracic midgut; AMG, abdominal midgut, and HG, hindgut The morphological forms are. 1, amastigotes; 2, stumpy promastigotes, 3, elongated nectomonad promastigotes; 4, short nectomonad promastigotes, 5, haptomonad promastigotes; 6, paramastigotes, 7, metacyclic promastigotes.

Handman 1992) On the other hand, both promastigotes and amastigotes possess their own lectin-like receptor which is, in some way, involved in the attachment (Hernandez 1986, Svobodova et al 1997a, b) Early studies emphasized also the role of direct binding of purified LPG and gp63 to CR3 receptor (Talamas-Rohana et al 1990, Russell & Wright 1988). However, this hypothesis was not confirmed using living promastigotes (Brittingham et al 1995, Rosenthal et al 1996) Similarly, direct binding of gp63 to fibronectin receptor also remains ambiguous (Mosser & Brittingham 1997) Although the biological significance of direct binding seems to be rather low in comparison with the efficient serum-dependent attachment, the observation that mice lacking C3 can be infected with *Leishmania* supported the role of direct binding *in vivo* (Mosser & Brittingham 1997)

The attachment is followed by phagocytosis the macrophage encloses the parasite cell and forms a parasitophorous vacuole where parasite surface membrane is surrounded by a host cell membrane which was originally on the surface of the phagocyte (reviewed by Bray & Alexander 1987, Chang 1983)

1 2 Survival and multiplication

During classical phagocytosis, lysosomes containing hydrolases fuse with phagosome containing the parasite (i e , parasitophorous vacuole) to form a phagolysosome The entry of *Leishmania* promastigotes is a somewhat different event, recent studies showed that promastigotes could inhibit phagosome-lysosome fusion due to an effect of LPG (Desjardin & Descoteaux 1997) Phagosome-lysosome fusion is re-established during the transformation of promastigotes to amastigotes, which is associated with the loss of LPG expression Then, amastigotes appear inside the hostile environment of the acid parasitophorous vacuole and have to survive macrophage microbicidal equipment including the lethal products of oxygen metabolism, lysosomal hydrolases and low pH

The survival of amastigotes is enabled not only by passive resistance to macrophage cell actions, but also by active influence on the host cell One of these parasite-induced changes is an impairment of macrophage Ca^{2+} homeostasis *Leishmania* spp, like other intracellular pathogens, can induce the elevation of Ca^{2+} in the host cell (Eilam et al 1985) Therefore, the parasite alters the macrophage Ca^{2+}-dependent signaling mechanisms and the response to extracellular stimuli, which affects macrophage functions like the respiratory burst and the MHC class II expression (reviewed by Olivier 1996) *Leishmania* spp can also enhance the viability of the host cell it was shown that *L donovani* infection prevents macrophage apoptosis The enhanced macrophage viability facilitates the spread of infection as well as the uptake by the sandfly vector by increasing number of host cells available for parasitation (Moore & Matlashewski 1994)

The main killing mechanism of macrophage which threaten leishmanias is the **oxidative burst**, i e , the generation of reactive oxygen intermediates (O_2 and H_2O_2) by the NADPH oxidase system *Leishmania* spp posses only negligible amounts of enzymes catalase and glutathione peroxidase which can inactivate the resultant elements of the burst (Meshnick & Eaton 1981) However, oxidative burst can be impaired in another fashion Firstly, as mentioned above, *Leishmania* can enter macrophages without eliciting oxidative burst Secondly, the parasite LPG is a powerful inhibitor of the activation of protein kinase C (PKC) (McNeely et al 1989, McNeely & Turco 1990) PKC is involved in the regulation of several cellular functions, including the enzymatic induction of oxidative burst As the consequence of a signal (rapid increase of intracellular Ca^{2+} levels), PKC is redistributed from the macrophage cytosol to the plasma membrane where it is further activated to its enzymatic function LPG was shown to inhibit both of these events It prevents the binding of PKC to membranes as well as inhibits the activity of membrane-bound form of this enzyme (Giorgione et al 1996) Thirdly, as shown by Sørensen et al (1994), purified gp63 also strongly inhibits the oxidative burst in monocytes And additionally, if the oxidative burst for all that is induced, the

glycan part of *Leishmania* LPG (McNeely & Turco 1990) and proteophosphoglycan (Ilg et al 1995) function as oxygen metabolites scavengers in promastigotes and amastigotes, respectively

The other recognized effector mechanism for the killing of *Leishmania* spp inside macrophages is the **production of nitric oxide (NO)** by the enzyme NO synthase (NOS) The cellular targets of NO toxicity are various enzymes of glycolysis and respiratory metabolism as well as trans-membrane transport systems of parasites (Mauel & Ransijn 1997) Proudfoot et al (1995, 1996) described that the synthesis of NO can be inhibited by *Leishmania* glycoinositolphospholipids (GIPLs) and phosphoglycan part of LPG GIPLs, the major glycolipid class and prominent surface antigens of *Leishmania* parasites, are structurally related to the anchor of LPG and are expressed at constant levels in both developmental stages (McConville & Blackwell 1991, Schneider et al 1993)

Both GIPLs and LPG can also provide a surface shield against **lysosomal hydrolases** (Chang 1993, Opat et al 1996) Alternatively, the resistance to lysosomal enzyme degradation is due to a direct inactivation of enzymes by excreted factors, i e LPG and phosphoglycan released by parasites (El-On et al 1980) or by the change of the intralysosomal pH (Coombs 1982)

Amastigotes do not proliferate immediately after the entry into macrophages It was shown *in vitro* that the proliferation of *L donovani* amastigotes do not start during the first 3–5 days of the infection (Pearson et al 1983, Eslami & Tanner 1994) The multiplication is followed by rupture of parasitized cell and the liberated amastigotes are ingested by other macrophages In comparison with wealth of information about promastigotes binding to macrophages, very little is known about the mechanism of entry of amastigote stages During macrophage attachment, amastigotes encounter different environment than did promastigotes before (early vs acute inflammation) It is, therefore, not surprising that amastigotes generally require for entry other receptors than promastigotes, mainly the receptor for Fc domain of immunoglobulins (Guy & Belosevic 1993, Peters et al 1995), although the involvement of LPG was also described for *L major* (Kelleher et al 1995)

1 3 Modulation of the host immune response

The *Leishmania* – macrophage interactions influence the host immune response and have a strong impact on either the spread or the suppression of the disease Activated macrophages, natural killer (NK) cells and T lymphocytes play a crucial role in the host defence against *Leishmania* spp The activation state of these effector cells is dependent on the availability of stimulatory and inhibitory cytokines Not surprisingly, *Leishmania* are able to alter their production and, in this way, affect a protective immune response of the host

The fundamental point to establish control of intracellular infection is the macrophage activation by cytokines derived from $CD4^+$T helper (Th) lymphocytes This event has been intensively investigated in experimental infections of inbred strains of mice with *L major* (reviewed by Reiner & Locksley 1995) The central finding evolved from the model was that resistance to the disease (spontaneous healing of the lesions) is related to the preferential expansion of Th1 cells while susceptibility involves the preferential expansion of Th2 cells Th1 and Th2 cells are differentiated from naive Th precursors in response to cytokines such as IL-12 and IL-4, respectively Th1 and Th2 cell subsets differ in the production of cytokines and, subsequently, in their functions Th1 cells mediate cellular immunity, effective against intracellular pathogens (activation of macrophages and cytotoxic $CD8^+$T-cells), while Th2 cells are responsible for humoral immunity, unprotective in this case (reviewed by Lohoff et al 1998, Lehmann & Alber 1998, Bogdan & Rollinghof 1999)

Leishmania spp counteract the development of Th1 immune response by modulation of the macrophage cytokine production In particular, they suppress IL-12 production which negatively affects Th1-cell expansion In addition, they stimulate macrophages for the induction of transforming growth factor-β (TGF-β) and IL-10 which suppress NK cells and macrophage effector functions (reviewed by Bogdan & Rollinghof 1998, 1999)

Leishmania spp. can also circumvent the induction of a protective Th1-cell response by impeding the presentation of antigens by MHC (major histocompatibility complex) class II. Without signals from antigen-presenting cells, Th1 cells could not be efficiently activated. De Souza-Leao et al. (1995) found that *L. amazonensis* amastigotes are able to internalize MHC class II molecules into parasite lysosomes (called megasomes) where they are degraded by cysteine proteases. However, it is not known yet whether this mechanism is sufficient enough to protect parasites. The interaction between antigen-presenting cells and Th cells is stabilized by CD4 molecules on the surface of Th cells. It was shown that gp63 from *L. major* and *L. donovani* cleaves CD4 molecules (Hey et al. 1994). This protective mechanism, however, is still ambiguous as it is not clear how gp63 could reach the surface of Th cells. The other factor impeding the host immune defence is a complex glycocalyx of amastigotes (containing mainly GIPLs) which protects the parasites from proteolytic attack as well as it masks the surface peptides of intact parasites. Therefore, only very few peptide epitopes are available for loading on the MHC molecules.

2. Factors affecting Leishmania *virulence*

In parasitic diseases, virulence is not the own characteristic of the parasite, but it is a complex process determined by the interactions of multiple parasite and host factors. According to Chang (1993), virulence is caused by **immunopathogenic determinants** of the parasite on one side and host **immunologic determinants** on the other side. Chang (1993) also distinguished the term 'pathogenicity' (or infectivity) which denotes an ability of the parasite to invade host using its various intracellular and surface molecules (**invasive determinants**). Therefore, pathogenicity is a prerequisite for leishmanial virulence. It is assumed that parasite invasive and immunopathogenic determinants are different molecules, regulated independently, and the disease symptoms as a manifestation of virulence depend on up- or down-regulation of all the three mentioned determinants.

The virulence of *Leishmania* isolates is, conventionally, compared by inoculation of animals with the same parasite numbers and by evaluation of disease symptoms. Most often, skin lesions and hepatosplenomegaly are checked in cutaneous and visceral disease forms, respectively. The assessment of virulence is based on the number of parasites found in infected organs and/or the size of the organs measured at different periods of the infection.

As the virulence is strongly influenced by host immunity and genetic susceptibility (Liew & O'Donnell 1993, Tanner 1996, Blackwell 1996), the animals used for experimental infections should be inbred, of the same age and sex (Giannini 1974, Sempervivo et al. 1981a, Neal 1984). Evenly important is the site of inoculation (Nabors et al. 1995) and conditions of *Leishmania* cultivation, like the medium and the growth phase of parasites used for inoculation (Giannini 1974, Neal 1984). It is generally believed that only the metacyclic promastigotes from stationary-phase cultures are able to infect vertebrates (Franke et al. 1985, Mallinson & Coombs 1986, Da Silva & Sacks 1987, Sacks & Da Silva 1987, Grimm et al. 1991). Thus, the actual assessment of *Leishmania* infectivity and virulence could be made, and difference among various strains and even cloned lines of the same strain could emerge, only if the above experimental conditions are controlled.

The diversity in *Leishmania* virulence in nature has not yet been elucidated. First reports concerning this topic came from field studies carried out in Central Asia (former USSR), where foci of zoonotic cutaneous leishmaniasis are caused by *L. major* with *Rhombomys opimus* as the main reservoir (see the Part IV). Kellina et al. (1981) found that field isolates from gerbils and humans were composed of clones displaying various degrees of virulence for mice and hamsters. Later, however, the avirulent forms have been identified as two separate species, *L. turanica* and *L. gerbilli* (reviewed by Strelkova 1996). More recently, different strains and clones of *L. peruviana* and *L.*

braziliensis which show reproducible differences in virulence have been obtained from Peru and Colombia (Dye 1992, Blackwell 1992, Dujardin et al 1995, Davies et al 1997) *L peruviana* is the causative agent of Andean cutaneous leishmaniasis ("uta") It is endemic in Andean valleys, where it forms small populations isolated by numerous natural barriers Dujardin et al (1995) found that the insulated nature of the habitat and decreased gene flow between separated populations contributed to formation of strong chromosomal polymorphism which is linked with phenotype variability, particularly the lesion type in patients and the virulence *in vitro* Also the experiments with *L tropica* suggest a role for natural parasite-related virulence determinants Isolates from patients with cutaneous lesions displaying different clinical patterns differed similarly in mice infections (Ebrahimzadeh & Jones 1983) and strains from cutaneous, viscerotropic and visceral human isolates differed in disease progression in mice and hamsters (Lira et al 1998)

In laboratories, parasites maintained by serial transfers in axenic cultures are under a strong artificial selective pressure In such conditions the quickly dividing forms of less virulent parasites are favoured and mutations affecting virulence are tolerated It is, therefore, suggested that a common mechanism occurring in laboratory strains *in vitro* is a gradual adaptation to culture conditions and/or selection for certain subpopulations that outgrow others present in the original heterogeneous population (Handman et al 1983, Segovia et al 1992, Camara et al 1995)

The loss of infectivity and/or virulence for host in long-term cultures was frequently observed (Adler 1961, Neal 1964, Ebert et al 1979, Giannini et al 1981, Kutish & Janovy 1981, Grimaldi et al 1982, Sempervivo et al 1981b, Handman et al 1983, Nolan & Herman 1985, Grimm et al 1991, Segovia et al 1992) Avirulence of parasites attenuated in the process of cultivation correlates with the loss of their ability to transform into metacyclic forms (Kallinikova et al 1992) On the other hand, promastigotes from long-term cultures could differ from virulent first-passage promastigotes in larger size and vigorous growth *in vitro* (Nolan & Herman 1985, Sempervivo et al 1981b, Grimm et al 1991)

It must be admitted that parasites maintained exclusively as amastigotes by serial passages in laboratory animals (or in macrophage-like cell cultures) are not threatened by avirulence, but they have a decreased capacity to function as promastigotes and to grow in culture Therefore, only frequent alternate passages in mammalian host and cultures could yield *Leishmania* capable of morphological and physiological transformations known to occur in the natural life cycle (Sempervivo et al 1981b)

In some cases, the loss of virulence is reversible Katakura & Kobyashi (1985) obtained a more virulent line of promastigotes from a less infective original strain after 15 serial passages in mice A single passage through susceptible animals resulted in increased virulence of parasites in less virulent clones producing delayed footpad lesions (Da Silva & Sacks 1987, Marchand et al 1987, Shankar et al 1993)

Avirulent parasites obtained by prolonged *in vitro* cultivation or produced on purpose by mutagen treatment are often used in research A comparison of these avirulent variants with the virulent ones is a useful method to study the phenotypic repertoire associated with the character of virulence In this way, one can obtain information about factors necessary for *Leishmania* survival in macrophages (Nolan & Herman 1985, Katakura 1986, Marchand et al 1987), about *Leishmania* surface antigens (Handman et al 1983, Ayesta et al 1985, Saraiva & Andrade 1986, Greenblatt et al 1985, Sanyal et al 1994) and about important **molecular determinants of virulence** like LPG and gp63 (see the Part VI), cysteine proteinases (Mottram et al 1996), GIPLs (McConville & Blackwell 1991), heat shock proteins Hsp100 (Hubel et al 1997) and Hsp70 (Kantengwa et al 1995) or Ca^{2+}-ATPase (Lu et al 1997)

PART VI.

The role of main surface molecules of *Leishmania* in the life-cycle

1. The structure and developmental regulation of LPG and gp63

As was shown above, molecules on the cell surface are crucial for the survival of *Leishmania* parasites both within the host and the vector. This chapter briefly summarizes main results of investigations concerning lipophosphoglycan (LPG) and the glycoprotein of 63 kDa (gp63). Although there are other important surface and excreted molecules like the gene B protein, GIPLs, proteophosphoglycan, acid phosphatase or protein kinases, it is clear that LPG and gp63 are exceptionally significant (reviewed by Turco & Descoteaux 1992, Moody 1993, Coombs & Mottram 1997). Both molecules are glycosylphosphatidylinositol (GPI)-anchored glycoconjugates constituting a major part of the dense glycocalyx covering the entire surface of promastigotes.

LPG is composed of four distinct structural domains: GPI-anchor, a conserved hexasaccharide core, a linear array of phosphorylated oligosaccharide repeat units and a saccharide cap (for details see Turco & Descoteaux 1992). LPGs from different *Leishmania* species and different developmental stages exhibit structural variations. During metacyclogenesis, the development of infective metacyclic forms is accompanied by about two-fold elongation of the molecule due to the increase in the number of repeat units, and concomitantly, a subtle compositional changes in these units (Sacks et al. 1990). These changes are suggested to be involved in the regulation of the attachment and detachment of promastigotes in the sandfly gut. The resulting metacyclic promastigotes display markedly enhanced resistance to both complement-mediated lysis and macrophage killing mechanisms (reviewed by Sacks 1992). Amastigote stages generally do not express LPG, the only described exception is *L. major*. However, the amastigote LPG of *L. major* is structurally and antigenically different from the LPG of promastigotes (Glaser et al. 1991, Moody et al. 1993)

The interspecific polymorphism in LPG structure is expressed in the type and number of oligosaccharide side chains branching from the conserved backbone repeat units. The most complex LPG with high number of side chains was found in *L. major* (McConville et al. 1990), while little or no substitutions occur in *L. donovani*, *L. mexicana* and in the *Viannia* subgenus (Thomas et al. 1992, Ilg et al. 1992, Muskus et al. 1997).

Gp63 has been identified as a zinc metalloproteinase, active on a wide range of protein substrates (Etges et al. 1986, Chaudhuri & Chang 1988, Bouvier et al. 1989) and with a substrate-dependent pH optimum (Ip et al. 1990, Tzinia & Soteriadou 1991). This molecule is ubiquitous among *Leishmania* promastigotes (Bouvier et al. 1987, Etges 1992) and is also present in amastigotes, although in lower levels (Button et al. 1989, Schneider et al. 1992, Bahr et al. 1993, Streit et al. 1996). In *L. mexicana*, most amastigote gp63 is confined to the flagellar pocket (Medina-Acosta et al. 1989) or to amastigote lysosomes called megasomes (Bahr et al. 1993, Ilg et al. 1993). On the other hand, surface location of the gp63 was found in amastigotes of *L. major* and *L. chagasi* (Pimenta et al. 1991, Streit et al. 1996).

2. LPG and gp63 as a virulence factors

Association of gp63 with parasite virulence has been reported by various authors. The increase of gp63 expression correlates with the development of infective metacyclic promastigotes (Kweider et al. 1987). Decreased amount and proteolytic activity of gp63 was found in avirulent parasites in comparison with the virulent ones (Chaudhuri & Chang 1988, Wilson et al. 1989, Santos-Gomes & Abranches 1996, Seay et al. 1996) and the transfection of avirulent strains with a gp63 gene has increased parasite infectivity for macrophages (Liu & Chang 1992, McGwire & Chang 1994, Chakrabarty et al. 1996). In other systems, however, the main role of LPG in parasite virulence was highlighted (Handman et al. 1986, McNeely & Turco 1990, Frankenburg et al. 1992). Decreased

virulence of *L. major* and *L. donovani* after chemical mutagenesis was found to correlate with the decrease in expression and change of LPG structure, while the expression and proteolytic activity of gp63 remained unchanged (Elhay et al. 1990, Shankar et al. 1993, Cappai et al. 1994).

This controversy may reflect the natural variability and plasticity in the relative importance of these two virulence factors. Indeed, Chakrabarty et al. (1996) showed that in two virulent strains of *L. donovani* the contributory roles of gp63 and LPG differ either in recognition or in the rate of internalization into macrophages. In their experiments, preblocking of macrophage receptors with either gp63 or LPG affected the entry of the one or the other virulent strain, respectively.

The cross-substitution of these two virulence factors can be imagined with respect to their suggested roles in the vertebrate host (summarized in Table 2). Both LPG and gp63 have been implicated in crucial steps of *Leishmania*-host interactions, i. e., the influence on monocyte migration, avoidance of complement attack, attachment to macrophages and protection from degradation in macrophage phagolysosomes. Some of the functions remain to be confirmed and some may be species-specific, however, it is clear that these molecules accomplish very similar functions in a different manner. Such diversity in evading strategies and multiplicity in molecules which are involved in the evading mechanisms may be, in fact, a main qualification for a successful survival of the parasite.

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Effect of insect hormone, 20-hydroxyecdysone on growth and reproduction in mice

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Abstract The effects of 20-hydroxyecdysone (20-E) on growth and reproduction following daily intraperitoneal injections have been studied in juvenile and adult mice of both sexes The injections contained 0 1, 0 5 and 1 0mg of 20-E, which correspond to final doses from 2 8mg g ' to 100mg g ', they were administered over a period of 30 days The effects on growth were evaluated by recording the daily increments of the living mass In addition, the effects on female reproduction were determined by vaginal smears, while the effects on male reproduction were evaluated by size of seminal vesicles and by the content of sperm in caudal epididymis It was found that 20-E enhanced growth in the groups of female juveniles but not in the male juveniles In the adults, 20-E caused increased growth in both males and females Due to pronounced cyclicity in female reproduction, the effects were more apparent in the males These results, obtained by injections of the purified compound, confirm previous conclusions regarding the anabolic-like action of ecdysteroids, which were reached principally by feeding the animals with a drug prepared from ecdysteroid-containing plants Despite its anabolic-like effects on growth in adult male mice, and in contrast to the action of true steroidal androgens, 20-E inhibited growth of seminal vesicles and decreased sperm production These effects of 20-E have been considered as potentially antiandrogenic The profound disruption of sexual heat cycles in adult female mice provides strong presumptive evidence for the qualification of 20 E, and perhaps other ecdysteroids as well, into the category of various phytoestrogens The possibility that 20-E could play a role as an essential sterolic vitamin is discussed

Growth, reproduction, anabolic effect, antiandrogenic effect, 20-hydroxyecdysone, mice

INTRODUCTION

Ecdysteroids (ES) are polyhydroxylated, 6-keto, D-7 sterols, were originally discovered in 1965 during the search for invertebrate moulting hormones (see reviews by Sláma & Lafont 1995) They are also present in plants, where they can occur in extremely high concentrations At present we know that ES can cause a plethora of pharmacological, vitamin-like effects in vertebrates (see reviews by Simon & Koolman 1989, Sláma & Lafont 1995) However, the true biological status of ES – whether they are insect hormones, defensive secondary compounds of plants, or essential vitamins of vertebrates – remains to be defined. Pharmacological preparations based on ES content have already been commercialized in several countries due to their anabolic, tonic, antidepressive, adaptogenic and rejuvenating properties (Sláma 1993).

The growth-stimulating anabolic effects of the most common ES, 20-hydroxyecdysone, were first observed in mice as early as 1969 by Hikino et al Since 1975, Syrov et al described the general anabolic action of ES in mice, rats, and other animals, using ES extracted from certain Asiatic plants (Syrov 1984, Syrov and Kurmukov 1975, 1976, Syrov et al 1975) According to Sergeev et al (1991) anabolic effects of ES isolated from plants were not associated in mice with adverse androgenic,

antiandrogenic or thymolytic side effects, in contrast to the effects of the true anabolic vertebrate steroid hormones The data on anabolic effects of ES collated to date, have been mostly related to rodents, but Koudela et al (1995) recently reported the profound growth-stimulating action of 20-hydroxyecdysone in birds (Japanese quails)

To date, scant information is available on ES effects with respect to vertebrate reproduction Prabhu & Nayar (1974) tested the possible estrogenic or antiestrogenic activity of ES in adult female mature white rats They found neither hormonal activity In adult male rats, Mirzaev & Syrov (1992) observed enhanced sexual activity, although the effect was only temporary In adult male mice, possible growth stimulating effects of 20-hydroxyecdysone were studied in comparison with those of a true vertebrate anabolic steroid, metandrostenolone (Chermnykh et al 1988) It appeared that both these sterolic compounds produced some anabolic effects, but only in association with the temporary training Finally, Bandara et al (1989) reported that 20-hydroxyecdysone had a spermicidal effect on human sperm

The data on the anabolic action of ES in vertebrates are mostly related to feeding experiments, using pulverized roots, green parts or seeds of ES-containing plants as food additives The disadvantage of such feeding experiments is that the effects of ES have been combined with a number of other biologically active, secondary plant substances present in the preparation For our research we used injections of purified 20-hydroxyecdysone to reinvestigate its effects on growth and reproduction in juvenile and adult mice of both sexes The effects have been compared with those of true anabolic vertebrate sex hormones, which were recently published by Bronson et al. (1996)

MATERIALS AND METHODS

Animals

Juvenile male and female mice (n=48, initial living mass m=10 g) and the sexually mature male and female mice (n=40, m=25 g) were used for the experiments The animals were placed in cages according to their age, living mass and sex, each cage containing either 5 or 6 mice In this way we obtained 4 basic experimental groups, these are juveniles of both sexes and adults of both sexes, with a further 4 subgroups in each basic group All the experimental and control animals were kept in one breeding room at 22 °C (± 2 °C) under 14/10h light/dark photoperiodic illumination Food and water was supplied *ad libitum*

Administration of 20-hydroxyecdysone

The ES compound 20 hydroxyecdysone (20E) was a natural product extracted and purified from the seeds of an Asiatic plant *Leuzea carthamoides* Iljin, using the procedures described by Slama et al (1996) The compound was a 96% pure 20E, with 4% contamination predominantly by other, biologically active, minority ecdysteroid compounds The 10% ethanolic stock solution of 20E (2 mg/ml) was prepared by dissolving 200 mg 20E in 10 ml of ethanol with subsequent dilution with distilled water to 100 ml Further dilutions were made only with water Each day the animals were injected intraperitoneally with 0 5 ml of the tested solutions, the daily dosages being adjusted to 0 1, 0 5 and 1 0 mg of 20E To prevent possible bias, all animals were subject to the same degree of manipulation, thus the control animals received daily injections of 0 5 ml of water The animals were weighed immediately after injections and manipulation limited to one minute maximum per animal, to respect ethical requirements for working with animals After 30 days of the experiment, the animals were humanely sacrificed

Data collection and data analysis

The blood samples were collected directly from the hearts of the sacrificed mice The weight of separate organs (heart liver, kidney, spleen, and testes) was determined on a Sartorius balance at a sensitivity range of 1 mg The amount of spermatozoa in the lower part of the epididymis (cauda epididymidis) was determined using a Burker counting chamber The cauda epididymis was dissected, separated from the rest of the epididymis and placed into 1 ml Eppendorf tubes After the addition of 1 ml of warm Tyrodes solution (38 °C, Sigma) the amount of spermatozoa was counted in the left and right epididymis separately The results are based on average values from the left and right organ

The data on relative increase of the living mass was calculated from the actual daily increments divided by the initial weight of the individual The linear model (Systat 5 01 for MS-Windows) was used for statistical verification of differences in relative daily increases of body mass or sperm counts among individuals in the group as well as

among different groups of experimental animals The data obtained in the adults, however, often revealed nonlinear growth curves, thus the Kruskal-Wallis one-way analysis of variance was used to evaluate the differences between the separate experimental groups

Vaginal smear test

Vaginal smears were taken during the last 20 days always between 9 00 and 10 00 a m They were stained for 3 min with May-Grunwald solution, then for 10 min with diluted Giemsa solution The separate oestral phases were determined according to light-microscopic investigation of the cell types Proestrus I Proestrus II, Oestrus, Metoestrus I, Metoestrus II, Dioestrus For comparison and better identification of the respective cell types, oestrus in another group of experimental females was artificially induced (not included in the results) using hormones PMSG and HCG

RESULTS

Effects of 20-E on growth of juvenile mice

As the best criterion for possible anabolic action of 20-E the average increments of the living mass in the course of 33-day growth period in postnatal mice were selected We have found profound sexual differences in the action of 20-E in immature mice Fig 1 shows that the compound does not stimulate growth in juvenile males throughout the whole range of daily dosages from 0 1 (group M1), 0 5 (group M2) to 1mg (group M3) Surprisingly, we found a slight inhibition of growth in this juvenile male group, which is more apparent after 15 days of the experiment According to General Linear Model statistics (GLM, Systat 5 01), the $F_{3\ 48}=2.057$, $P<0.118$.

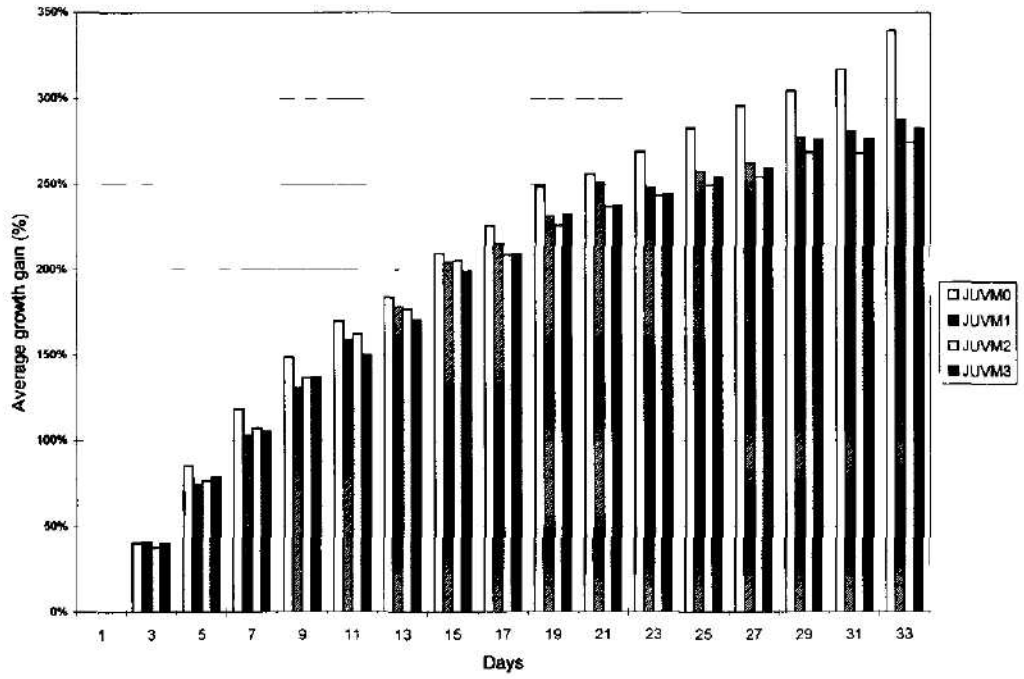

Fig 1 Juvenile male mice Effects of daily intraperitoneal injections of 20-E on living mass during 33-day period of growth JUVM0 – controls, JUVM1 – 0 1 mg, JUVM2 – 0 5 mg, JUVM3 – 1 mg of 20-E per injection

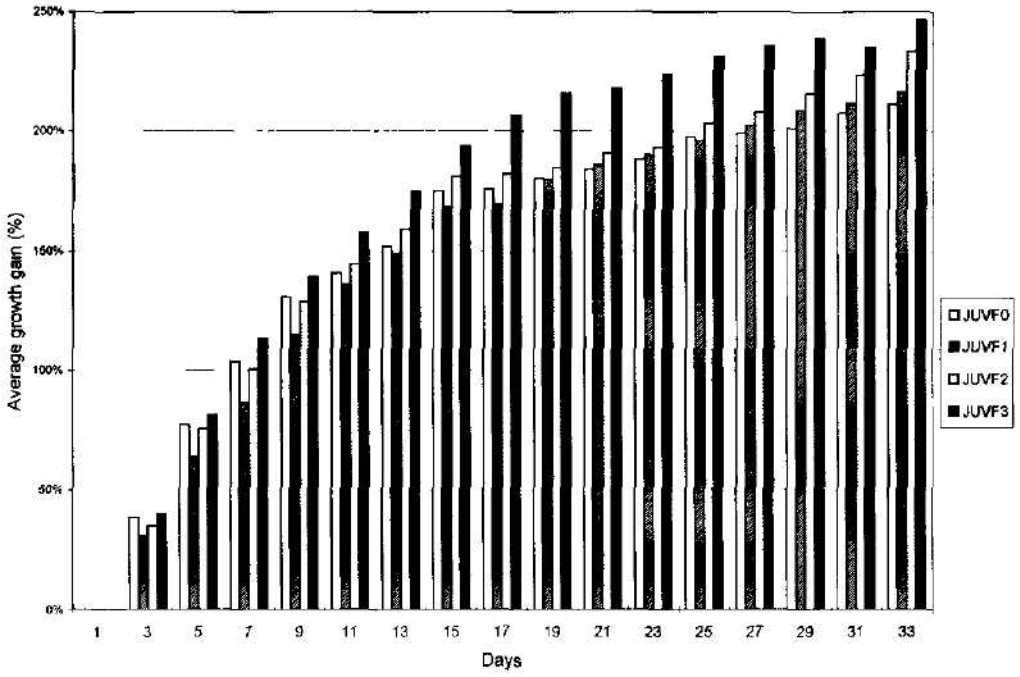

Fig 2 Juvenile female mice Effects of daily intraperitoneal injections of 20-E on living mass during 33-day period of growth

A substantially different situation has been found in the groups containing female juvenile mice In this case, as shown in Fig. 2, the 20-E treatment exerted remarkable stimulation of growth rate, with a dose-dependent character. The differences in relative increments of the living mass between the control groups and the groups receiving daily 20-E are statistically highly significant ($F_{3,48} = 6.853$, $P<0.001$).

Effects of 20-E on growth of adult mice

In adult male mice, peritoneal daily injections of 20-E positively stimulated growth. This is documented in Fig 3 which shows a regular, dose-dependent, increase of the living mass. GLM statistical analysis does not entirely evaluate the data presented in Fig. 3 ($F_{3\ 32} = 0.835$, $P<0.485$), as Fig. 3 shows growth curves which are not exactly linear. However, according to the Kruskal-Wallis one-way analysis of variance, the differences between the control and experimental groups are highly significant ($F_{3\ 32} = 26.954$, $P<0.0001$). We may thus conclude that the androgenic, hormone-like, growth stimulating influence of 20-E in adult male mice has been clearly confirmed. The described stimulation of growth by 20-E in adult male mice also occurs in adult females, as shown in Fig. 4. However, due to rather pronounced cyclical changes which are normally associated with female reproduction, the data clearly shows a nonlinear, cyclic distribution. The Kruskal-Wallis, one way ANOVA analysis of the data in Fig. 4 has shown a very significant differences ($F_{3,\ 32} = 21.859$, $P<0.0001$) between the control and experimental groups.

The finding of growth-stimulating effects of 20-E injections in adult mice of both sexes is quite consistent with previous data obtained by oral administration of 20-E in the diet. These results also agree with previous conclusions that 20-E or ecdysteroids in general are indeed responsible for the growth-stimulating effects of crude drugs prepared from various ecdysteroid-containing plants.

The comparison of living mass increments caused by 20-E in adult versus juvenile female mice revealed relatively smaller increments (related to body size) in the adults (see Fig. 5 for comparison). This relative difference between the immature and adult stages is most likely to be related to general physiological differences in the rate of growth; that of adult mice occurs at the flattened, terminal part of the common growth curve.

In order to obtain more data on possible physiological differences between the separate experimental groups, the mice were subjected to several biometrical investigations after autopsy at the end of experiments. We evaluated average body mass, length of the body, length of the tail and feet. There were considerable variations in these biometrical values between the groups as well as between separate specimens of a given experimental group. Statistical analysis of the data mostly indicated low confidence in what was really due to the effects of 20-E. Only in the groups of adult males did we find a statistically significant increase of the body mass which was dependent on the dose of administered 20-E, see Fig. 6. The animals in the group that received daily 1 mg of 20-E increased average body mass from 41.7 to 44.9 g, which shows a 106.7 % increase.

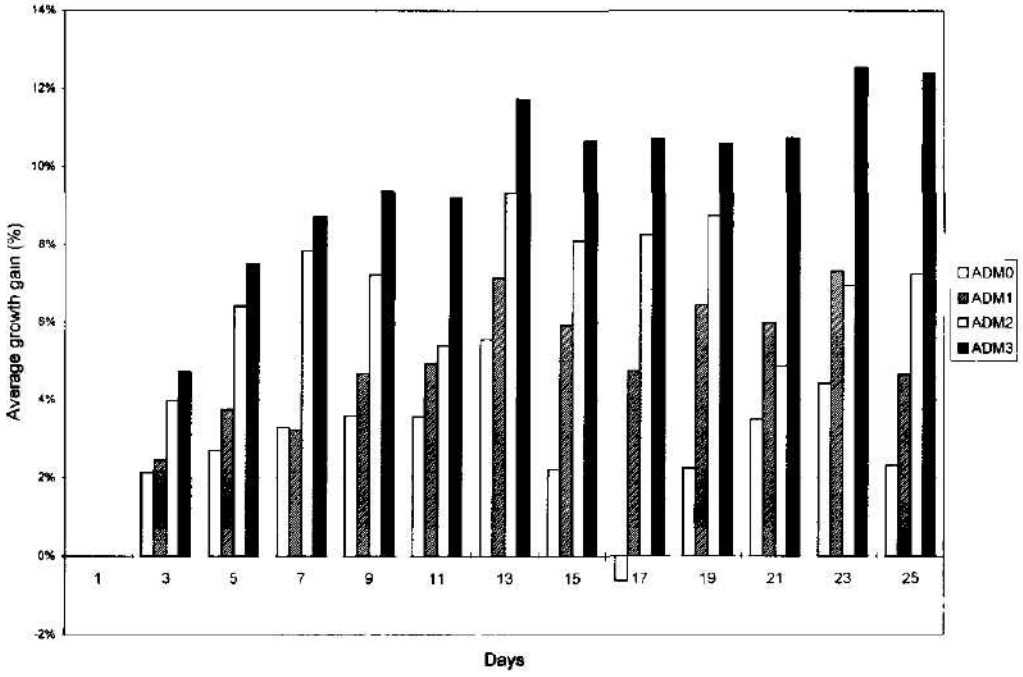

Fig. 3. Adult male mice. Effects of daily intraperitoneal injections of 20-E on living mass increments during 25-days of adult life.

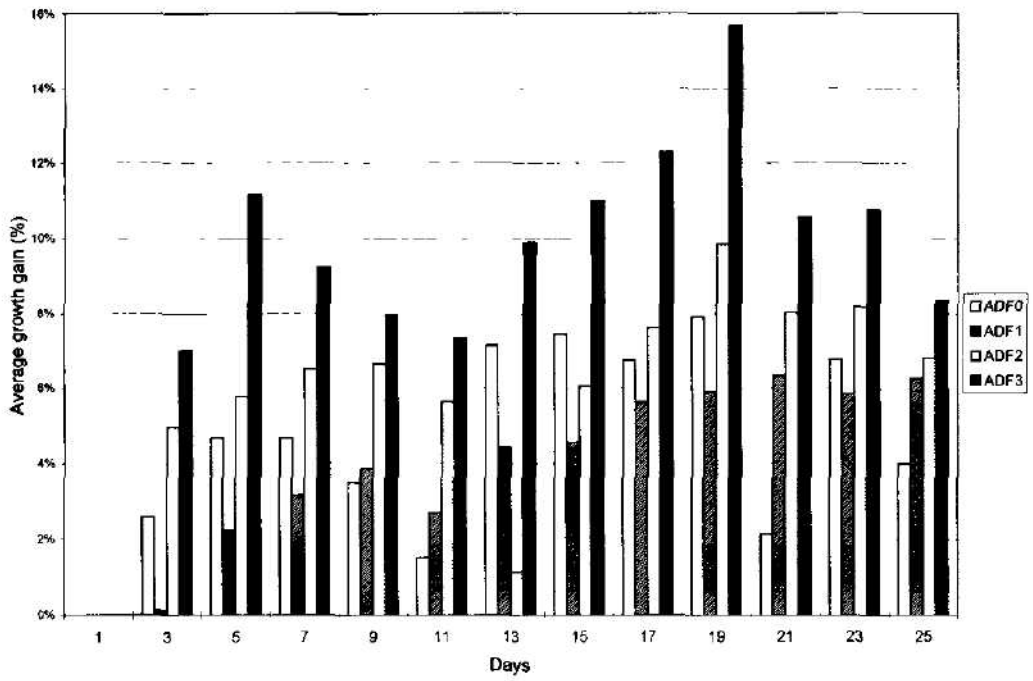

Fig. 4. Adult female mice. Effects of daily intraperitoneal injections of 20-E on living mass increments during 25-days of adult life.

After the autopsies we also determined the mass of certain internal organs (liver, spleen, kidney and heart). In most cases we did not reach statistically significant differences between the control and experimental groups. There was one exception, however, which was related to the size of the liver in adult males. The average mass of liver in the control group of adult males was 1.92 g (±0.08 SD). The livers of the experimental groups amounted to 2.04 (±0.28), 2.07 (±0.03) and 2.11 (±0.37) in the groups that received 0.1, 0.5 and 1mg of 20-E, respectively. This shows that adult males which received daily 1mg of 20-E had enlarged mass of the liver, an increase of 109.8% in comparison to the control adult males.

Effects of 20-E on sperm production

We used two essential criteria for evaluating the effects of 20-E on reproduction in males:

a) average number of sperm in the caudal epididymis, to assess the influence on sperm production, and;

b) size of seminal vesicles and viability of spermatozoa after ejaculation. The results revealed a highly significant, dose-dependent, negative effect of 20-E on sperm production ($F_{3,11} = 34.634$, $P<0.0001$) and a negative effect on the size of the seminal vesicles ($F_{3,17} = 19.889$, $P<0.0001$). This has been shown in Fig. 7A and 7B, which also shows that this inhibitory action of 20-E does not apply to juvenile males (Fig. 7A, 7B).

Effects of 20-E on oestrous cycles in adult females

The selected groups of adult females appeared to be rather heterogeneous with respect to the length of individual heat cycles and also with respect to average length of the cycle in separate experimental groups. This is documented in Fig. 8, which does not show any constant deviation in individual phases of the oestrous cycle by 20-E. The search for possible dose-dependent deviations of oestrus did not reveal significant differences. However, there was one common feature: all three investigated experimental groups of females had a more or less prolonged period of the whole oestrous cycle. In this case the difference from the control group was highly significant ($P<0.0001$). Our failure to find a constant difference in the separate phases of the oestrous cycle were apparently due to the fact that 20-E produced irregularities of the cycles in both directions. The cycles were either shorter (0 to 1 day) or longer (3 to 4 days), while the average was 2 days in the control groups. One particular female receiving 20-E showed even 9 days of prolonged vaginal cornification. In conclusion we can state that 20-E produces irregularities in oestrous cycles and, as shown in Fig. 8, it prolongs average duration of the cycle.

After termination of the experiment (after 30 days) we examined the ovaries for the presence of yellow bodies (corpora lutea). The ovaries of the control females, adult or juvenile controls, showed the presence of multiple yellow bodies. In contrast, the females receiving 20-E had only 2 or 3

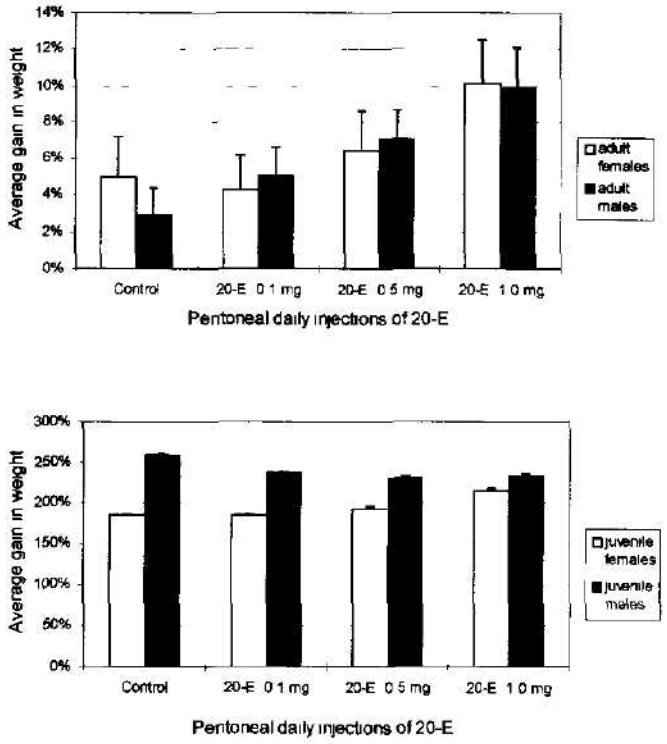

Fig. 5 Comparison of the average daily increments of living mass in 4 investigated groups of mice, the juvenile males and females and the adult males and females

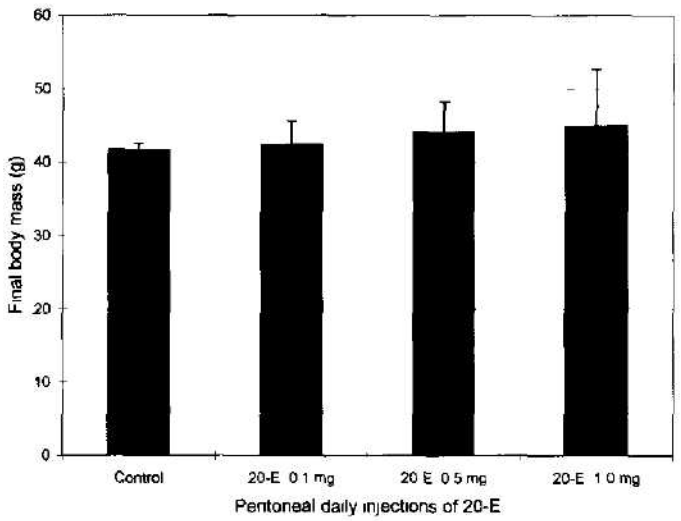

Fig 6. Adult male mice Effect of 20-E on final size of the body after termination of the experiment after 25 days

yellow bodies in each ovary, without a clear-cut dependence on the dose. This may also help to explain the heterogenity, irregularity and asynchrony in separate oestrous phases.

DISCUSSION

In summary, our results show that daily peritoneal injections of 20-E increased the rate of growth in juvenile females but not in juvenile males. Moreover, 20-E also stimulated the rate of growth in adult mice of both sexes, but inhibited the production of sperm in males and caused disturbances of oestrous cycles in the females.

Hikino et al. (1972) found that intraperitoneally administered 20-E in mice was rapidly distributed into various internal organs, quickly transferred into intestinal lumen via the gall bladder and then excreted. Sláma & Lafont (1995) calculated that the range of physiologically effective concentrations of 20-E should be between 20 and 100 ppm, both in insects as well as in vertebrates. We have used doses of 0.1, 0.5 and 1.0 mg of 20-E per animal and day, which corresponds to the post-injection concentrations of 10, 50 and 100 ppm respectively in the juveniles (living mass 10 g) or, to the concentrations of 2.85, 14.2 and 28.5ppm in the adults (average living mass 35 g).

The influence on growth, which we have induced with 96% pure 20-E in mice, is relatively smaller in comparison with that caused by true, hormonal anabolic androgens (cf. Bronson et al. 1996). In our experiments, the virtual symptoms of anabolic action were only found in adult male mice. In this group we have obtained a 107 6% increase of the living mass with daily injections of 1mg 20-E per animal. Koudela et al. (1995) obtained up to 120.4% increase of growth in Japanese quails which received the ecdysteroid-containing diet Our findings of increased size of liver in the treated males confirm the observations of Hikino et al.(1969) who used injections of 5–100mg of 20-E and cyasterone in mice for the first time. In our experiments we used 100–1000mg of 20-E, e.g up to 10-fold increase. The fact that we did not observe any symptom of intoxication confirms previous findings on the very low acute toxicity of 20-E (Sláma and Lafont, 1995). We have calculated that

the range of acute toxicity of 20-E in mice should be far beyond 28 mg.kg $^{-1}$.24 h^{-1} (adults) or 100 mg.kg $^{-1}$ 24 h^{-1} (young juveniles).

In spite of the accumulated data about anabolic action of ES (Syrov 1984), the hormonal nature of these effects in vertebrates can be questioned According to Sláma (1993) and Sláma & Lafont (1995), these invertebrate hormones in vertebrates play a biological role more akin to sterolic vitamins. Vitamins have no direct regulatory functions; their physiological effects are manifested in the case of their insufficiency It is known that vertebrates receive ecdysteroids in plant food (for review see Simon & Koolman 1989, Sláma & Lafont 1995). It has been recently found in Japanese quails that blood titres of 20-E correspond closely with the content of 20-E in their diet (Sláma et al 1996) We may thus conclude that if a vitamin-like nature of 20-E in vertebrates would really exist, it could only be elucidated by experiments on animals fed an axenic, ecdysteroid-defficient, diet Unfortunately, no such experiments have been undertaken to date.

Changes in size of seminal vesicles are routinely used as a criterion for the androgenic potency of steroid hormones and their bioanalogues (Clark & Barber 1994). Our results obtained with administration of 20-E in adult male mice show the opposite, that is antiandrogenic effects on the growth

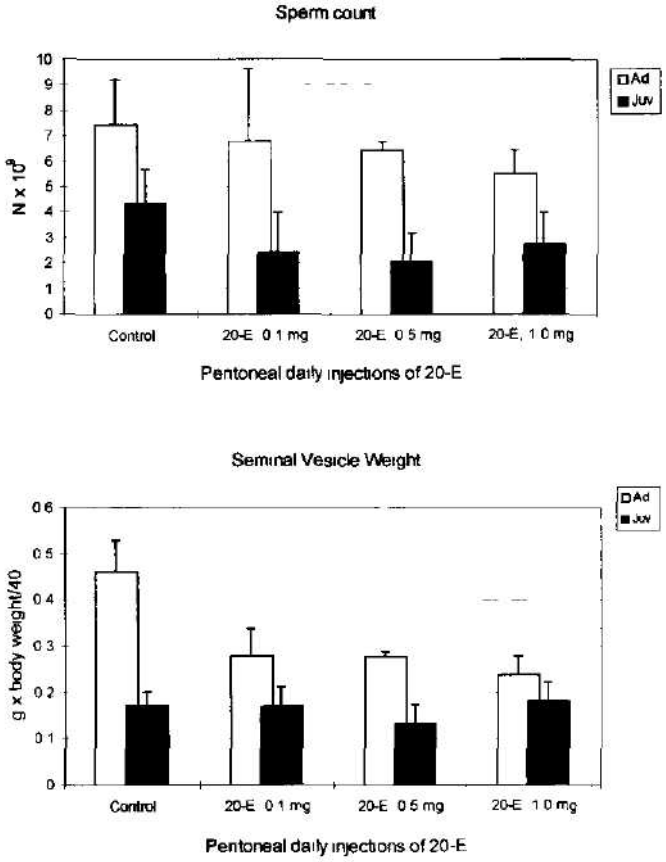

Fig 7 Adult male mice Effect of 20-E on sperm count above (A in the text) and size of seminal vesicles (B) (± SD) after termination of the 25-day assay period

of the seminal vesicles. The inhibitory action of 20-E on seminal vesicles and production of sperm occurred in spite of the enhanced growth of liver and of the whole body. The antiandrogenic-like effect of 20-E on seminal vesicles and on the amount of spermatozoa in the caudal epididymis has been less apparent in the groups of juvenile males, however, this may be due to the fact that their androgenic activity was not fully developed. The adverse effects of 20-E on sperm production in mice could be related to the observations of Bandara et al. (1989) who found spermicidal activity of 20-E in human sperm. The described dose-dependent, antiandrogenic effects of 20-E are in large contrast with the effects of methyltestosterone and other androgenic hormones (Clark & Barber 1994). We conclude, therefore, that the anabolic effects of ES in vertebrates should be understood as operating in terms of a different principle to the androgenic physiological principle.

The effects of 20-E on female reproduction do not preclude the possibility of hormonal interactions. Adult females of mice, rats and hamsters have a very short oestrous cycle which usually lasts 4 days (Lisk 1978). In addition to internal physiological factors, the cycles in mice are also influenced by social conditions and other external factors (Cinquetti & Rinaldi 1989). However, this was not the case in our experiments, where the tentatively estrogenic or antiestrogenic effects were positively due to administration of 20-E. The receptors of estrogenic hormones are widely opened to nonspecific interaction with a number of estrogen mimics which are commonly present in plants. These phytoestrogens include nonsteroidal molecules like isoflavones, furocoumarins, cyclic lactones, stilbenes and, what is more important, also polyhydroxylated triterpenoid molecules with

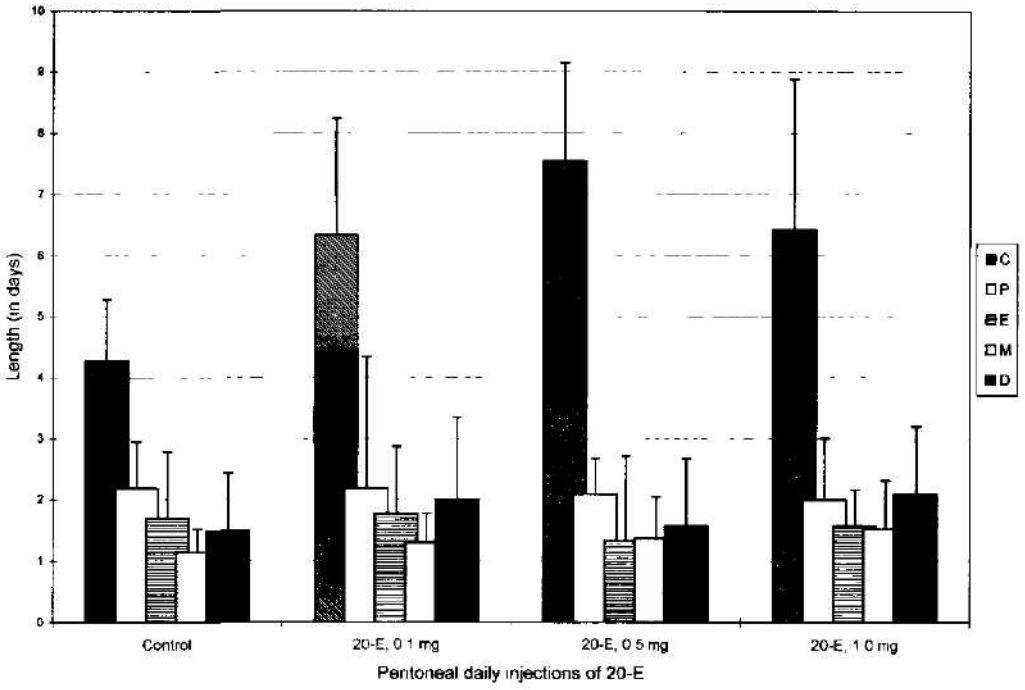

Fig. 8. Adult female mice. Effect of 20-E injections on duration of oestrus cycle. C – overall length of the cycle, P - prooestrus, E - oestrus, M – metoestrus, D – dioestrus. Error bars indicate SEM.

structural similarities to the ecdysteroids (for review see Sláma 1980). The most important structural requirement for a natural or synthetic estrogen mimic is the presence of two hydroxylic groups that should be positioned across the molecule exactly at the distance given by the 3-OH and 17-OH positions in estradiol. Some ecdysteroids fulfill this essential estrogenic structural feature and, therefore, they can theoretically qualify as phytoestrogens. The disturbances of sexual heat cycles, which we induced by administration of 20-E to adult female mice, show all symptoms of the well known hyperestrogenic syndrome, which occurs in various domestic animals after consumption of phytoestrogen-containing food (Sláma 1980).

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On the taxonomy and nomenclature of the Palaearctic Oedemeridae (Coleoptera)

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Abstract Several new synonyms, new combinations and status changes are established in the present paper concerning the following genera of the family Oedemeridae: *Calopus* Fabricius, 1775, *Chrysanthia* W Schmidt, 1844, *Nacerdes* Dejean, 1834, *Anogcodes* Dejean, 1834, *Opsimea* Miller in Reitter, 1880, *Probosca* W Schmidt, 1844, *Ischnomera* Stephens, 1832, *Indusclera* Švihla, 1980, *Chitona* W Schmidt, 1844 and *Oedemera* Olivier, 1789

Taxonomy, new synonymies, new combinations, status changes, Coleoptera, Oedemeridae, Palaearctic region

INTRODUCTION

This paper was written during the preparation of an article on the family Oedemeridae, which will be published in the forthcoming "Catalogue of the Palaearctic Coleoptera". The deadline for the publication of taxonomic and nomenclatural acts, which will be included in the Catalogue, is 31 December 1999. This forced me to publish these data in a separate paper, although I would prefer to include them in review-articles on particular taxa.

MATERIAL AND METHODS

This paper is based on the study of types and a considerable amount of additional material, as well as on critical revisions of original descriptions of the taxa

Abbreviations used in the text:
BMNH = British Museum of Natural History, London, United Kingdom, J Beard;
HNHM = Természettudomanyi Muzeum, Budapest, Hungary, O Merkl,
IJNC = collection of J Jeniš, Náklo, Czech Republic;
MNHN = Muséum National d'Histoire naturelle, Paris, France, C Girard,
NHMV = Naturhistorisches Museum, Wien, Austria, H Schonmann;
PMSL = Prirodoslovni Muzej Slovenije, Ljubljana, B. Drovenik,
ZMAS = Zoological Institute of AN, St. Petersburg, M G Volkovitsh

I am very obliged to all above mentioned colleagues for the kind loan of the type and other material.

The localities of the type material are quoted according to the original labels, while those of additional material are transcribed. If no references are given in the part Distribution, it is based on the material, deposited in author's collection

TAXONOMIC PART

Calopus serraticornis (Linnaeus, 1758)

Cerambyx serraticornis Linnaeus, 1758: 395
Calopus pretneri G. Müller, 1929: 63, **syn. n.**

TYPE MATERIAL EXAMINED Syntype, "Sveto Brdo, Velebit, 15.–16 VI 25, E Pretner" (PMSL), designated here as lectotype

REMARKS. No differences including those, mentioned in the original description, between the lectotype of *C. pretneri* and the material of *C. serraticornis* from Central and Northern Europe, was found

Chrysanthia chalcochroa chalcochroa Fairmaire, 1892

Chrysanthia chalcochroa Fairmaire, 1892: 159.
Chrysanthia tauricola Pic, 1920: 5, **syn. n.**

TYPE MATERIAL EXAMINED *Ch. chalcochroa*: syntypes. "Syrie, Amanus, C. D. 1891", 1 male, 1 female; "Syrie, Akbès", 1 male, male specimen from Akbès is designated here as lectotype, the others as paralectotypes; *Ch. tauricola*: holotype, male, "Taurus, ?Karsanlı? (unreadible)" (all MNHN)

DISTRIBUTION. Southern Turkey: Bey Daglari Mts., Toros Daglari Mts., Nur Daglari Mts.
REMARKS. No essential differences were found between *Ch. chalcochroa* and *Ch. tauricola*.

Chrysanthia chalcochroa holzschuhi Pardo Alcaide, 1972 stat. n.

Chrysanthia holzschuhi Pardo Alcaide, 1972: 214

TYPE MATERIAL EXAMINED Paratype, male, "Anatolien, Prov. Antalya, Alanya 1.–7.6.1970, leg. C. Holzschuh" (VSPC)
ADDITIONAL MATERIAL EXAMINED Turkey, Prov. Antalya: Sagırın, 5.vi.1989, G. Gillerfors lgt., 1 ex., 3.vi.1989, S. Lundberg lgt., 1 ex., Beskonak, 31.v.1989, S. Lundberg lgt., 1 ex.; S. of Beskonak, 24.v.1978, K. & S. Wellschmied lgt., 2 ex. (all VSPC)

DISTRIBUTION. Southern Turkey: southwestern slopes of Toros Daglari Mts. in the Antalya Bay.
REMARKS. The subspecies differs from the nominotypical one only by the partly orange coloration of pronotum and by yellow anterior tibiae and orange bases of the femora. Morphological characters including the aedeagus are the same in both subspecies.

Chrysanthia varipes planiceps Kiesenwetter in Schneider et Leder, 1878 stat. n.

Chrysanthia planiceps Kiesenwetter in Schneider et Leder, 1878: 256
Chrysanthia turcica Pic, 1899b: 15, **syn. n.**

TYPE MATERIAL EXAMINED *Ch. turcica*: holotype, female, "Turquie" (MNHN).

REMARKS. This subspecies differs from the nominotypical one only by entirely metallic coloured femora. Other morphological characters were found to be the same, including the form of the aedeagus, some of them are rather variable (form of the head and pronotum). *Ch. varipes planiceps* occurs in northern Turkey, Georgia and southern Russia (Caucasus Mts.), while the nominotypical subspecies is so far known from Croatia, Bosnia, Yugoslavia, Macedonia, southern Bulgaria and Greece.

Chrysanthia cyprica Pic, 1920 stat. n.

Chrysanthia varipes var *cyprica* Pic, 1920 5

TYPE MATERIAL EXAMINED Syntypes, "Cyprus, Nicosia" 2 males, lectotype and paralectotype is here designated (MNHN)

REMARKS. This species is closely related to *Ch. varipes* Kiesenwetter, 1861, however it differs from the latter by the apex of the aedeagus, which is slightly but distinctly curved ventrad. So far known only from Cyprus.

Chrysanthia flavipes Reitter, 1889

Chrysanthia flavipes Reitter, 1889 266.
Chrysanthia oralis Fairmaire, 1892: 158, **syn. n.**
Chrysanthia oralis var *semicuprea* Pic, 1920: 5, **syn. n.**

TYPE MATERIAL EXAMINED *Ch oralis*, syntypes, "Syrie, Akbès" 1 male, 1 female; "Syria, Amanus, 1891, C D.", 4 females (all MNHN). One female from Amanus is here designated as lectotype, the other specimens as paralectotypes: *Ch flavipes*, syntype, male, "Siria, Akbes", designated here as lectotype (HNHM).

REMARKS. Both *Ch. flavipes* and *Ch. oralis* do not differ one another by any important character.

Chrysanthia viridissima (Linnaeus, 1758)

Cantharis viridissima Linnaeus, 1758 403.
Chrysanthia viridissima var *diversipes* Pic, 1932: 30, **syn. n.**

REMARKS. The new synonym is only a coulour abberation within the variability of the species.

Nacerdes (s str.) *melanura* (Linnaeus, 1758)

Cantharis melanura Linnaeus, 1758 403
Nacerdes melanura var *zoufali* Reitter, 1907. 161, **syn. n.**

REMARKS. The new synonym is only a colour abberation within the variability of the species.

Nacerdes (*Asiochroa*) *bicostata* (Lewis, 1895) stat. n.

Xanthochroa watterhousei var. *bicostata* Lewis, 1895: 436

TYPE MATERIAL EXAMINED Syntypes: male,"Japan, Nara, G Lewis / Xanthochroa bicostata, Type (Lewis's handwriting)", designated here as lectotype; "Fukushima, 26 vii –29.vii 81, G Lewis / Xanthochroa bicostata, Type (Lewis's handwriting)" female, designated as paralectotype (all BMNH)

REMARKS. *N* (*A*.) *bicostata* is closely related to or conspecific with *N.* (*A.*) *katoi* (Kôno, 1932), type material of which was not yet studied.

Anogcodes seladonius seladonius (Fabricius, 1792)

Necydalis seladonia Fabricius, 1792: 352.
Anoncodes viridipes W Schmidt, 1846: 116, **syn. n.**
Anoncodes meridionalis Costa, 1852: 10, **syn. n.**

REMARKS. Type material of the newly synonymised species was not found Both species were cited as synonyms of *A. ruficollis* (Fabricius, 1781), however according to the original descriptions they are conspecific with *A. seladonius seladonius*.

Anogcodes geniculatus (Schmidt, 1846)

Anoncodes geniculata W. Schmidt, 1846: 99.
Anoncodes geniculata var. *basalis* W. Schmidt, 1846, **syn. n.**
Lethonymus difformis Marseul, 1857, **syn. n.**
Anoncodes versicolor Chevrolat, 1873: 205, **syn. n.**
Nacerdes (*Pachychirus*) *tschitscherini* Semenow, 1895: 248, **syn. n.**
Anoncodes signaticollis Pic, 1898: 2, **syn. n.**
Anoncodes difformis var. *bodemeyeri* Pic, 1911: 170, **syn. n.**

TYPE MATERIAL EXAMINED: *N. tschitscherini*, holotype, female, "Sarat" (ZMAS); *A. difformis* var. *bodemeyeri*, holotype, female, "Asie-turquie, Tschakit-Thal, v. Bodemeyer" (MNHN)

REMARKS. This species possesses not only striking sexual dimorphism, but females are strongly variable in their coloration, what caused, that this species was described so many times. Type material of other here synonymised taxa was not examined, however colour forms agreeing with the original descriptions are deposited in the author's collection.

Anogcodes fulvicollis (Scopoli, 1763)

Cantharis fulvicollis Scopoli, 1763: 43
Anoncodes media Gredler, 1863: 295, **syn. n.**
Nacerda fulvicollis var. *therondi* Méquignon, 1948: 78, **syn. n.**

REMARKS. The new synonyms are only colour forms within the variability of the species.

Anogcodes coarctata croceiventris (Motschulsky, 1859) stat. n.

Anoncodes croceiventris Motschulsky, 1859: 453
Nacerda coarctata var. *manciurica* Magistretti, 1939: 159, **syn. n.**

REMARKS. This subspecies differs from the nominotypical one only by the entirely yellow coloration of the abdomen in female, while only the last abdominal segment is more or less paler in the nominotypical subspecies. *A. coarctata croceiventris* occurs in Primorye, Manchuria and Korea, where it overlaps at some localities with the nominotypical subspecies, and in Sakhalin and Japan exclusively.

Opsimea ventralis Miller in Reitter, 1880

Opsimea ventralis Miller in Reitter, 1880: 225
Oedemera quadrinervosa Reiche, 1862: 296, nec Latreille, 1804: 12
Stenaxis parallela Fairmaire, 1892: 158, **syn. n.**

TYPE MATERIAL EXAMINED: *S. parallela*, syntypes, "Syrie, Akbès", 1 male, 1 female, male is designated here as lectotype, female as paralectotype (MNHN)

REMARKS. *Stenaxis parallela* does not differ significantly from the original decription of *Opsimea ventralis*.

Probosca (*Proboxantha*) *marginata* Walker, 1871

Probosca marginata Walker, 1871: 17
Probosca notaulthorax Pic, 1906: 26, **syn. n.**

REMARKS. *P. marginata* was described from Cairo. Walker's type material was destroyed, however the original description agrees very well with the material of *P. notatithorax* at my disposal, including a specimen from Egypt: Gara, 4.vii.1935, J. Omer-Cooper lgt.

Ischnomera caerulea (Linnaeus, 1758)

Cantharis caerulea Linnaeus, 1758: 403
Oedemera quadrinervosa Latreille, 1804: 12, **syn. n.**

REMARKS. Type material *Oedemera quadrinervosa*, described from France, was not found. The original description of this species agrees only with *Ischnomera caerulea* within the oedemerid fauna of France.

Chitona (*Dolichopyga*) *tristis* (Faldermann, 1837) comb. n.

Nacerdes tristis Faldermann, 1837: 140

REMARKS. Type material of this species was not found, however according to the original description it belongs to the relationship of *Ch.* (*D.*) *fucata* (Faldermann, 1837), below which it is situated also in the Faldermann's paper.

Indasclera himalaica Švihla, 1980

Indasclera himalaica Švihla, 1980: 50.
Indasclera himalaica godawariensis Švihla, 1997: 437, **syn. n.**

MATERIAL EXAMINED: C Nepal, Godawari, 14.–17.v.1992, I. Jeniš lgt., 1male (IJNC)

REMARKS. The additional examined specimen from the type locality of *I. himalaica godawariensis* have metallic coloured legs, what was the distinguishing character of the nominotypical subspecies, so that the new synonymy can be established.

Oedemera (*Oncomera*) *antoinei* (Pic, 1932)

Oncomera antoinei Pic, 1932: 30
Oncomera antoinei var. *mimeuri* Pic, 1949: 74, **syn. n.**

REMARKS. The type material of the described variety was not found. According to the original description, compared with material at my disposal, this variety is within the limits of the variability of coloration of the species.

Oedemera (*Oncomera*) *murinipennis* Kiesenwetter, 1859

Oedemera murinipennis Kiesenwetter, 1859: 192
Oedemera (*Oedemerella*) *paganettii* Stolz, 1915: 91, **syn. n.**

TYPE MATERIAL EXAMINED: Syntypes, "Kreta, Paganetti", male and female, male is designated here as lectotype, female as paralectotype (NHMV)

REMARKS. *O. paganettii* does not differ both from the original description of *O.* (*Onc.*) *murinipennis* and from large material of this species at my disposal. Both species were described from Crete.

NOMENCLATORIC NOTES

The following names were published as varieties or forms of the infrasubspecific rank (ICZN 1985, Art 45 g (ii) (1)) and never used for a species or subspecies before 1985 Hence, the provisions of the Code do not apply to them and the names cannot be used for the purposes of synonymy or homonymy

Asclera (Nacerdasclera) sibirica var *analis* Munster, 1921 9
Asclera (Nacerdasclera) sibirica var *androchroa* Munster, 1921 9
Chrysanthia oralis var *differens* Pic 1899b 15
Anoncodes amoena var *concolor* Mulsant, 1858 134
Nacerda (Lethonymus) difformis var *differens* Pic, 1899a 115
Asclera incostata var *ruficornis* Pic, 1926 19
Asclera incostata var *obscurimembris* Pic, 1926 19
Oncomera femorata f *treneri* Hartig, 1926 160

My paper (Švihla 1986) was by myself and secondarily by other authors erroneously dated with the year 1985 When I checked the complete issue of the journal (not only the reprint), I found on the cover "Published in 1986" (in Czech) Thus, all the taxa described in this paper have to be cited with the combination of 1986

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BOOK REVIEW

YUKAWA J & MASUDA H (eds) 1996 **Insect and Mite Galls of Japan in Colors** Tokyo Zenkoku Noson Kyoiku Kokai, 826 pp (in Japanese) ISBN 4-88137-061-8 C3645, Price not given

Only a few books in the world literature are devoted to the interesting group of animals that cause galls on plants One of them appeared in Japan, edited by Junichi Yukawa, the eminent international entomologist specializing mainly in the study of gall midges (Cecidomyiidae, Diptera) He is the Professor of Entomology at the Entomological Laboratory, Faculty of Agriculture, at Kyushu University, Kyushu, Japan He initiated the collaboration of 21 Japanese researchers, each of them the specialist of a particular insect or mite group, on this large project The co-editor of this book, Hisashi Masuda, is an outstanding hymenopterologist and is concerned in the study of gall wasps (Cynipidae) He confirmed the host plant alternation of many species and connected sexual and asexual generations which had been thought to be two different cynipid species In this book Japanese researchers, under the leadership of Junichi Yukawa and Hisashi Masuda, present color photographs of galls, showing the richness of galling organisms, and summarize knowledge of gall-making organisms occurring in Japan The book is divided into two parts In the first part, after introductory information, 353 colour photographs of galls caused on various host plants by gall-making organisms are compiled into 40 plates, printed on coated paper Colour photographs of galls are arranged alphabetically according to the host plant genera in botanical system, starting with Pteridophyta and Coniferae Each photograph is identified by the combination of a Latin letter and an Arabic number, and with the popular name in Japanese The color photographs are of high quality and show various shapes of galls or damage to different plant organs (leaves, needles, stems, flowers, buds, fruits and seeds) Several galls are shown in longitudinal sections to show larval chambers and developing larvae, or some galls are captured by the camera just at the moment when the adult insect emerges from the gall in which it had developed The second part of the book, including additional and explanatory comments, tables and several lists, is divided into eight parts **Part I** includes accounts of the galls in short articles about each insect or mite species causing a gall, which includes references Gall-making organisms are dealt with under a sequence of indicative letter-numbers and in the same order as they are arranged in the pictorial part of the book **Part II** is a general account devoted to a historical review of the study of insect-induced galls, the cecidology, to problems of gall terminology galling mechanisms, the adaptive significance of galling, and the shape of galls and their position on plants Galling organisms are arranged according to a zoological system Shorter paragraphs are devoted to several groups of invertebrates and longer paragraphs to the insect groups Thysanoptera, Hemiptera, Coleoptera, Hymenoptera, Diptera and Lepidoptera The last sub-chapter deals with phytocecidia, which are galls caused by viruses, mycoplasmas, bacteria and fungi This part is illustrated by drawings showing the shape and organisation of galls and the morphological character of representatives of various groups **Part III** deals with arthropod communities centered upon galls Here there are explanations of the terms used in the book, such as parasitoid, predator, inquiline, cecidophage, honeydew collector, symbiote and successor **Part IV** includes methods of collection, observation and rearing of galls **Part V** is devoted to thirteen gall-making organisms belonging to various animal groups which are the most important gall-causing species in Japan They are serious pests of agricultural plants and forest trees Each species is dealt with in detail **Part VI** contains references, grouped into separate parts, according to subjects, viz review of Japanese literature on gall-causing organisms in general, review of foreign literature devoted to this subject, references about galling mechanisms, and references relevant to each gall-causing group of organisms This part is written both in Japanese and in other languages **Part VII** includes eleven tables summarizing data in the book from various points of view It contains a list of host plants and gall-causing species arranged according to Japanese names, with Latin names and with letter-numbers referring to color photos, a list of gall-causing insects and mites according to taxonomic groups and a review of the number of species belonging to all gall-causing groups in Japan Based on this summary, 1,423 species of gall-causing organisms occur in Japan The family Cecidomyiidae of the order Diptera (Insecta) is the most numerous group including 628 species (44%) followed by the family Cynipidae of the order Hymenoptera (Insecta) with 180 species (13%), the family Eriophyidae (Acari) with 171 species (12%) and the family Aphididae (Homoptera, Insecta) with 136 species (10%) Other insects groups are not so much abundant The last of the tables (Table VII–11) is the most important for the user who does not understand Japanese It includes the scientific (Latin) names of the gall-making organisms and their host plant mentioned in the explanation of the color plates, together with additional comments **Part VIII** contains five indexes, firstly the scientific (Latin) names, secondly Japanese names of plants and galls, thirdly Japanese names of gallers and related organisms, fourthly Japanese terms and fifthly general foreign terms The book "Insect and mite galls of Japan in Colors" is a very interesting and important work because it is the first review of knowledge about gall-causing organisms occurring in the south-eastern part of the Palaearctic region It is written in Japanese, which will limit its general use

Marcela Skuhrava

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The layer of musculi contrahentes in insectivores (Mammalia)

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Abstract The layer of the musculi contrahentes occurs in adult mammals in different modifications. It is defined by its situation in the autopodium in the ontogeny as well as postnatally. In adult individuals of the species *Sorex araneus* (Soricidae), we demonstrated by dissection the presence of a fibrous plate throughout the palm at the site of the layer studied. In the species *Erinaceus europaeus* (Erinaceidae) we found only vestigial muscles corresponding to the contrahent muscles. In the *Talpa europaea* (Talpidae) and *Chrysochloris asiatica* (Chrysochloridae) no layer of mm. contrahentes was demonstrated by the dissection. From an outline comparing our findings with data from the literature concerning the formation of the layer studied in further insectivorous animals it follows that the pattern of the mm. contrahentes layer is rather connected with the position of a particular species in the zoological system than with the function of the fore limb.

Musculi contrahentes, muscle layers of autopodium, Insectivora

INTRODUCTION

Within the scope of studying the morphology and development of muscle layers of the mammal autopodium, we considered the layer of the musculi contrahentes (seu adductores, seu mm. flexores breves medii). In accordance with the general scheme of the pattern of muscle layers in the mammal autopodium (Bardeleben 1890, Čihák 1969, Dylevský 1971), the characteristic feature of the layer of the mm. contrahentes is its situation between the layer of the mm. lumbricales connected with tendons of the m. flexor digitorum profundus and ramus palmaris profundus nervi ulnaris, which separates the contrahent muscles from the short deep flexors situated more deeply in the palm. This typical position of the layer studied in the autopodium and its inervation from the r. palmaris prof. nervi ulnaris served as a criterion for the identification of its particular muscles in our work.

The mm. contrahentes layer occurs in adult mammals in different species to the different extents and in different arrangements, sometimes it is quite lacking (Cunningham 1878, Young 1880, Mivart 1881, Dobson 1882, Leche 1900, Ribbing 1909, Kajava 1911, Forster 1916, Greene 1935, Howel 1936, Reed 1951, Haines 1955, Štěrba 1957, 1958, Vaughan 1959, Jouffroy 1962, Trnková & Dylevský 1969, 1971, 1972, 1984, 1994, Norberg 1970, 1972, Trnková 1973). The mm. contrahentes layer was also observed in the foetal period of the human hand ontogeny. The process of the reduction of the extent of this layer in the course of the human autopodium development was described by Čihák (1963, 1967, 1969), Grim (1972), Mrázková (1983). Different ways of the development of the mm. contrahentes layer in the ontogeny of certain mammal species are described by Trnková & Dylevský (1969, 1972, 1984, 1994, 1996) and Trnková (1974).

Various shapes or possibly absence of the layer studied in adult individuals of different species remains unclear. By comparing the results of the study of the mm. contrahentes layer in different particular mammal orders we try to find, whether these different shapes are connected with different

ways of using the limb and thus, whether it may be considered as an functional adaptation, or if it is possible to find a connection between the pattern of the layer and position of the relevant species in the zoological system considered in terms of phylogeny The work presents results acquired by the study of the mm contrahentes layer in insectivores.

MATERIAL AND METHODS

For the dissection of palm muscles, we used fore limbs of adult representatives of the order Insectivora as follows *Sorex araneus* Linnaeus, 1758 (Soricidae) 9 specimens, *Erinaceus europaeus* Linnaeus, 1758 (Erinaceidae) 4 specimens, *Talpa europaea* Linnaeus, 1758 (Talpidae) 4 specimens, *Chrysochloris asiatica* (Linnaeus, 1758) (Chrysochloridae) 1 specimen The material was fixed with 65% alcohol containing a slight amount of glycerol The dissection was mostly implemented with the help of a stereoscopic microscope

RESULTS

By the dissection we found that in *Sorex araneus*, under tendons of long flexors of fingers connected with lumbrical muscles, there is a fibrous plate throughout the palm, connected proximally to the fibrous tissue of the bottom of the canalis carpi and extended distally up to the level of metacarpophalangeal joints Its distal arcuate margins remind of a web by their shape This fibrous tissue layer

Tab I The pattern of mm contrahentes layer of Insectivora Explanations (1–7) the pattern of mm contrahentes layer described by authors (1) – Čihák (1963, 1968), (2) – Dobson (1882), (3) – Forster (1916), (4) – Haines (1955), (5) – Leche (1900), (6) – Reed (1951), (7) – Ribing (1909), * – species dissected, + – m contrahens exists, R – the rest of muscle, F – fibrous plate

Family	species	mm contrahentes digiti				
		I	II	III	IV	V
Solenodontidae	*Solenodon paradoxus* (2,5)	+	+			+
	Solenodon cubanus (2,5)	+	+			+
Tenrecidae	*Tenrec ecaudatus* (2,5)	+	+			+
	Setifer setosus (2,5)	+				+
	Microgale longicaudata (2)	+	+			+
Potamogalidae	*Potamogale velox* (2)	+	+			+
Chrysochloridae	**Chrysochloris asiatica* (2,5)					
Erinaceidae	**Erinaceus europaeus* (1)		R	R	R	
	Erinaceus sp (2,7)					
	Echinosorex gymnurus (2)					
Soricidae	**Sorex araneus*	F	F	F	F	F
	Sorex trowbridgii (6)					
	Crocidura sp (4)					
	Blarina brevicaudata (6)					
Talpidae	**Talpa europaea* (2,3,7)					
	Scapanus latimanus (6)					
	Scapanus sp (2)					
	Scalopus aquaticus (2)					
	Condylura cristata (2)					
	Neurotrichus gibbsii (6)	+				
	Desmana moschata (2,5)	+				
	Galemys pyrenaica (2,5)	+				

corresponds by its position in the palm to the mm contrahentes The r palmaris prof nervi ulnaris is extended under the thickened ulnar margin of the fibrous plate, which can be observed through the transparent layer (Fig 1a)

In *Erinaceus europaeus*, we found by the dissection, that under the layer of tendons of long flexors, there is a typical pair arrangement of the mm flexores breves profundi The ramus palmaris prof nervi ulnaris, extended along an arcuate way in direction of the thumb, was observed only up to the level of the half of the m flexor brevis profundus of the finger 4 Here, the nerve enters the muscle tissue and during its further radial continuation it perforates short deep flexors (Fig 1b) Parts of the mm flexores breves profundi situated palmarly from the nerve can be separated by fine dissection

In *Talpa europaea*, under tendons of long flexors of fingers, we found only vestiges of short deep flexors, onto which the r palmaris prof nervi ulnaris was situated We found neither muscular nor fibrous formation, which could correspond by its position in the autopodium to the mm contrahentes layer

During the dissection of limbs of *Chrysochloris asiatica* we found no formations, which could belong to the mm contrahentes layer Under a relatively thin ramus palmaris prof nervi ulnaris, we found only vestiges of short deep flexors

The comparison of our findings and data from the literature concerning the layer of the mm contrahentes in further species of Insectivora (including references) is summarized in the table 1

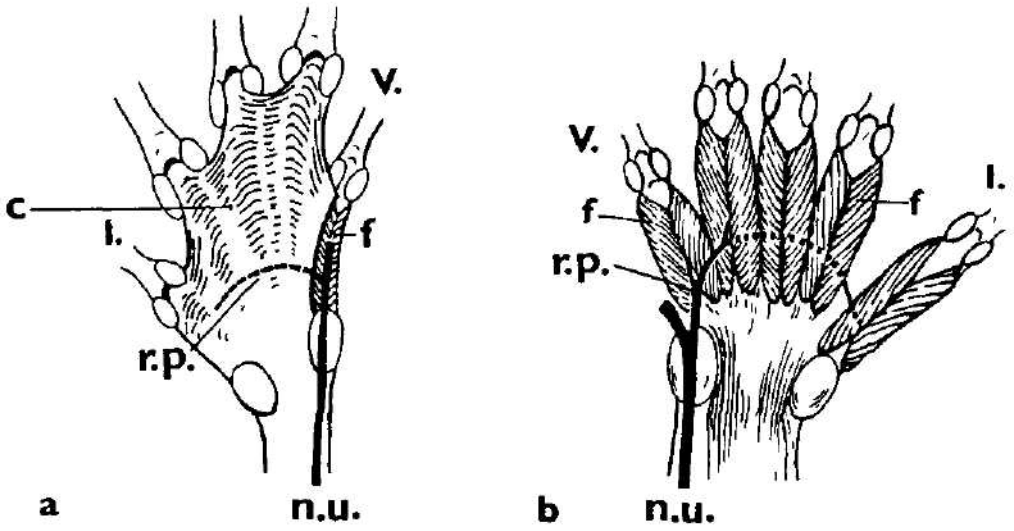

Fig 1 Scheme of the pattern of contrahent muscle layer in a – *Sorex araneus*, b – *Erinaceus europaeus* Explanations I and V – the first and fifth digits, c – contrahent muscle layer, f – mm flexores breves profundi, n u nervus ulnaris, r p – ramus palmaris profundus

DISCUSSION

The authors describing muscles of the autopodium in insectivores of the family Soricidae (Reed 1951, Haines 1955) mention no muscles falling into the layer of the mm. contrahentes. By detailed dissection, in the *Sorex araneus* (Soricidae), at the site of the assumed contrahent muscles, we demonstrated the presence of a fibrous plate throughout the width of the palm. The presence of this layer was also supported by its occurrence in the embryonal autopodium of this species (Trnková 1972, 1974, Trnková & Dylevský 1972).

Ribbing (1909) and Dobson (1882), in their description of muscles of different species of the genus *Erinaceus*, do not mention the mm. contrahentes. Čihák (1963, 1968, 1969) found that the r. palmaris prof. n. ulnaris in the *Erinaceus europaeus* perforates the deep flexors of the fingers 2, 3 and 4, tightly under the surface, which is in agreement with our findings. The portion of the muscle tissue situated palmarly from the extension of the nervus is considered by Čihák as a vestigial layer of the mm. contrahentes, which was attached to the layer of short deep flexors. We believe that this interpretation is correct.

Dobson (1882), Ribbing (1909) and Forster (1916) in the description of the autopodium of the species *Talpa europaea* (Talpidae) and Dobson (1882) and Leche (1900) in the *Chrysochloris asiatica* (Chrysochloridae), mention no muscles, which would belong to the layer of the mm. contrahentes. Our work supported the finding of these authors.

CONCLUSION

The comparison of all the data about the mm. contrahentes layer in insectivores supports our concept that the formation of this layer is not directly associated with the fore limb function. For example, we can see the same pattern of the layer in the terrestrial genera *Solenodon* and *Tenrec* as in the aquatic *Potamogale velox*. In the terrestrial insectivores of families Tenrecidae and Erinaceidae living in a similar environment, in the first case, three typical contrahent muscles are formed and, in the second case, the layer is either missing postnatally or only its vestiges can be observed. A more or less identical formation of the layer of the mm. contrahentes or possibly its absence in representatives of the same family rather indicates a connection with the taxonomic position of a particular species.

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In memoriam of Vladimír Jan Amos Novák

Vladimír Jan Amos Novák, DSc. unexpectly died on 29 September 1997 at the age of 78. He was born on 22 April 1919 in the family of a professor of geography at Charles University. After final examinations at a realgymnasium in Prague-Vršovice he enrolled, in the autumn of 1938, in the Faculty of Science of Charles University, taking up biology and geography. While the Czech universities were closed between 1939 and 1945 he took various occasional jobs. When World War II was over, V. J. A. Novák resumed his studies and at the end of 1945 passed state examinations in biology and geography. In 1946 he graduated with a RNDr. Degree in biology. From 1945 he worked in the Institute of General Zoology as an assistant and later lecturer under Prof. V. Breindl. In October 1948 he obtained a Cambridge University fellowship and spent ten months in the prestigious department of Prof. V. B. Wigglesworth, founder of modern physiology of insects. The visit profoundly influenced the scientific career of V. J. A. Novák. In February 1951 he went to the Soviet Union where he fell ill and spent many months in a Charkov hospital. On returning home and undergoing further treatment in Prague he began to work, in March 1953, as a researcher in the Institute of Biology of the Czechoslovak Academy of Sciences. In 1962 Dr. Novák joined the staff of a newly established Institute of Entomology, CSAS as a head of the Department of Insect Physiology. In 1956 he obtained a CSc. Degree, and the DSc. one in 1968. He was elected Corresponding Member of the Czechoslovak Academy of Sciences in 1972.

After 1975, becoming interested in the broader biological questions of animal evolution, he founded a Department of Evolutionary Biology at the Institute of Microbiology, CSAS. A Laboratory of Evolutionary Biology was set up in 1985 and V. J. A. Novák was its director till his retirement. Afterwards, for the rest of his life, Vladimír continued working intensively, publishing in the fields of evolutionary biology and social sciences. From his student years, throughout the War and later at the University, Vladimír studied the faunistics, systematics and, in particular, ethology of the social life of ants. In 1946–1948 he investigated, together with other members of Charles University's Institute of Zoology, the incidence of malaria and its vectors in southern Slovakia. However, it was his research on insects metamorphosis hormones which brought Vladimír international renown. He published several papers on the subject in prestigious journals (Nature, Journal of Embryology and Experimental Biology) during his visit of Cambridge, and afterwards he concentrated on it at the Institute of Biology in Prague. His own results as well as the collected findings of other authors appeared in his comprehensive monograph Insektenhormone (two editions in German, Publishing House of the Czechoslovak Academy of Sciences 1959, 1960), which has remained to this day one of the fundamental works in this field. The book was published again in two supplemented English editions (Methuen 1966, Chapman & Hall 1975), and made him one of the leading figures in the field

of metamorphosis and morphogenesis. During his work on this comprehensive book Vladimir Jan Amos Novák formulated some general hypotheses, such as those included in the so-called gradient-factor theory. That theory, which he kept modifying according to new findings and which he tried to bring up to the molecular level, had many supporters, but also many opponents even among V. J. A. Novák's students. Nevertheless, it had played a role in the subsequent direction of the study of insect hormones. Insect hormones and general questions of animal morphogenesis were the main topics of research in the Department of Insect Physiology of the Institute of Entomology. Vladimír Jan Amos Novák assembled there a very active team of young researchers interested in the new line of research, thus founding a school of thought which has since been represented by hundreds of important papers. Thanks to Vladimír Jan Amos Novák and his coworkers we now know relatively much about the functions of metamorphosis hormones, neurohormones, and about the role of hormones in diapause. The study of numerous questions concerning juvenoids and biologically active substances has produced applicable results. V. J. A. Novák's laboratory gradually became one of the top centres of this line of research. Over twenty scientists, Czech as well as from other countries, studied for their PhD degrees there, and many important visitors were received. In 1965, Vladimír organized an international Sympossium on Insect Endocrinology in Brno with star attendance. It was one of the milestones in insect physiology.

Vladimír was a born synthetist, used to assessing various aspects of questions and results and incorporating them into a broad context. The gradient-factor theory and new results of the research on insect metamorphosis and morphogenesis led him to studying the morphogenesis of live organisms in general, that is, to evolutionary biology, which became the focus of his work in the Department and later Laboratory of Evolutionary Biology. At first alone and then with co-autors he published papers on the phylogeny – ontogeny relatioship, principles of sociability, hormone theory, neoteny and its significance to evolution. Together with Academician Oparin he investigated general questions of the origins of life. The range of his activities concerning organisation of science was unusual. He organised many symposia, conferences and seminars, e. g. Evolution and Environment (Brno, 1961), Natural Selection (Liblice, 1978), Morphogenesis and Evolution (Plzeň, 1984), and Towards a New Synthesis (Prague, 1987). He was invited to congresses, lecture tours and research visits in European countries, Canada, China, Japan, and repeatedly to the USA.

In his last years, Vladimír Jan Amos Novák became interested in social sciences and published papers on various aspects of biology and philosophy, sociology, psychology, education, economics and politics. Novák's work in the broader context of evolutionary biology will be used and developed by his students and colleagues. His ideas concerning social sciences had better be evaluated by specialist in this field.

Awards in recognition of his work included Czechoslovak State Prize for advancement of insect endocrinology (1968), and Silver and Gold G. Mendel Plaques for merit in biology (1974, 1979). V. J. A. Novák was also active in popularisation of science. He published many articles in Czech Journals and newspapers and a book, The Unknown World of Insects (Prague, Orbis 1958)

There is no doubt that Vladimír Jan Amos Novák was an outstanding figure in international biology, a personality that is not easily forgotten. We miss the memorable debates with him on scientific and other issues. He was straightforward, patiently explaining his views and defending them with stubborn dignity. I do not remember him ever giving up an argument. Nevertheless, various problems were solved in those conversations, which sometimes gave rise to new ideas. Those who have known him will certainly remember him. And I believe that those who have never met him will appreciate his contribution to the development of modern physiology of insects and evolutionary biology.

Vladimír Landa

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